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(54) TRANSAMINASE AND USE THEREOF

(57) Atransaminase and a use thereof are provided. The transaminase has the amino acid sequences as shown in SEQ ID NO: 2 or 4, or has at least 80% identity to the amino acid sequences as shown in SEQ ID NO: 2 or 4, or has amino acid sequences which are obtained by the substitution, deletion or addition of one or more amino acids and have an the activity of an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the high stereoselective refers to the content of one of the stereoisomers being at least about 1.1 times that of the other. The transaminase can synthesize a chiral amine of R-configuration with a relatively high chiral purity, and is therefore suitable for the industrial use of the synthesis of chiral amines.

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Description

Technical field of the invention

[0001] The present invention relates to the field of synthesis of chiral compounds, and particularly to a transaminase and uses thereof.

Background of the invention

[0002] Chiral amines are ubiquitous in nature. They are common structural units in many important bioactive molecules, both synthetic and natural in origin. Chiral amines are a common structural motif in many drugs. Many chiral amines are also important chiral auxiliaries and chiral selectors. Therefore, the preparation of chiral amines is of significant economic importance.

[0003] At present, chiral amines are mainly prepared by means of chemical reduction, and amines with optical activity are prepared by using prochiral ketones. Catalyzed by Pd/C and quinine, a prochiral ketone reacts with formic acid and an ammonia or an organic primary amine to generate a chiral amine. Other researchers obtained a chiral amine through asymmetric amination and reduction of a prochiral ketone using a ruthenium complex as a catalyst (Renat Kadyrov et al. Highly Enantioselective Hydrogen-Transfer Reductive Amination: Catalytic Asymmetric Synthesis of Primary Amines. Angewandte Chemie International Edition. 2003, 42 (44), Page 5472 to Page 5474). The metal catalyst in such a reaction is a very critical factor and demands strict requirements on the metal catalyst. Also, it is necessary to carry out the reaction at high temperature and there are high requirements on operation devices. Additionally, the metal catalyst is expensive and an environmental pollutant (Ohkuma T et al. Trans-RuH (eta1-BH4) (binap) (1,2-diamine): a catalyst for asymmetric hydrogenation of simple ketones under base-free conditions. Journal of the American Chemical Society.2002, 124(23), Page 6508 to Page 6509).

[0004] An aminotransferase, also known as a transaminase, may catalyze an exchange process between amino and carbonyl groups of alpha-amino acids. An omega-transaminase is a transaminase capable of catalysing a transamination reaction using substrates other than alpha-amino acid. Omega-transaminase may effectively produce a chiral amine through stereoselective transamination using a variety of ketones as raw materials. The omega-transaminase has attracted more and more attention by researchers because of its use of relatively cheap substrates and its ability to produce highly pure products. It is expected that the potential of omega-transaminase can be fully applied toward the industrial production of chiral amines. However, there is still a need for further research and invention of this class of enzyme.

[0005] There is a demand for an omega-transaminase with high catalytic activity and stereoselectivity toward the R-configuration so that demand for chiral amine can be satisfied.

Summary of the invention

[0006] The present invention aims to provide a new transaminase and the uses thereof to satisfy demands of industrial production of chiral amines.

[0007] A transaminase or a modified compound, functional equivalent, functional fragment or variant thereof is provided according to an aspect of the present invention so as to achieve the purpose above. The amino sequence of the transaminase comprises a sequence selected from one of the following sequences: a) an amino acid sequence as shown in SEQ ID NO: 2 or 4; b) an amino acid sequence with at least 80% identity to the amino acid sequence as shown in SEQ ID NO: 2 or 4 and having the activity of an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the amino acid sequence is not the amino acid sequence encoded by a nucleotide sequence as shown in SEQ ID NO: 5 or 6; c) a protein which is derived from SEQ ID NO: 2 or 4 by subjecting the amino acid sequence as shown in SEQ ID NO: 2 or 4 to substitution, deletion or addition one or more amino acids, and having the activity of an omega-transaminase with high stereoselectiveR-configuration catalytic activity, wherein the amino acid sequence is not the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 5 or 6, wherein the high stereoselectivity refers to the content of one of the stereoisomers being at least about 1.1 times that of the other.

[0008] Further, the amino acid sequence of the transaminase is an amino acid sequence acquired by substituting leucine at the 38^{th} site of the amino acid sequence as shown in SEQ ID NO: 2 by isoleucine.

[0009] A nucleotide is provided according to another aspect of the present invention. The nucleotide encodes the transaminase or the modified compound, functional equivalent, functional fragment or variant thereof.

[0010] Further, the sequence of the nucleotide comprises a sequence selected from one of the following sequences: a) a nucleotide sequence as shown in SEQ ID NO: 1 or 3; b) a nucleotide sequence with at least 80% identity to the nucleotide sequence as shown in SEQ ID NO: 1 or 3 and encoding an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the nucleotide sequence is not the nucleotide sequence as shown in SEQ ID NO: 5 or 6; c) a nucleotide sequence capable of hybridizing with the nucleotide sequence as shown in SEQ ID NO: 1

or 3 under highly stringent conditions and encoding an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the nucleotide sequence is not the nucleotide sequence as shown in SEQ ID NO: 5 or 6, wherein the high stereoselective refers to the content of one of the stereoisomers being at least about 1.1 times that of the other.

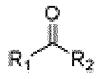
[0011] A recombinant vector is provided according to another aspect of the present invention. The nucleotide is effectively connected in the recombinant vector.

[0012] Further, the recombinant vector is pET22b-CM32 or pET22b-CM33.

[0013] A host cell is provided according to another aspect of the present invention. The foregoing recombinant vector is transformed or transfected into the host cell.

[0014] A method for synthesizing a chiral amine is provided according to another aspect of the present invention. The method includes the following steps: making a ketone compound, the transaminase or the modified compound, functional equivalent, functional fragment or variant thereof, pyridoxal phosphate, and an amino donor to react in a reaction system so as to obtain the chiral amine of R configuration.

[0015] Further, the ketone compound is



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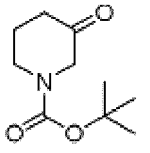
wherein R_1 and R_2 are independently C_1 to C_8 alkyl, C_5 to C_{10} cycloalkyl, C_5 to C_{10} aryl or C_5 to C_{10} heteroaryl; or R_1 and R_2 form a C_5 to C_{10} heterocyclic radical, a C_5 to C_{10} carbocyclic radical or C_5 to C_{10} heteroaryl with a carbon on a carbonyl group; heteroatoms in the C_5 to C_{10} heterocyclic radical and C_5 to C_{10} heteroaryl are independently selected from at least one of nitrogen, oxygen and sulfur; the aryl in the C_5 to C_{10} aryl, the heteroaryl in the C_5 to C_{10} heteroaryl, the carbocyclic radical in the C_5 to C_{10} carbocyclic radical or the heterocyclic radical in the C_5 to C_{10} heterocyclic radical is independently unsubstituted or is substituted by at least one radical of halogen, alkoxy or alkyl; preferably, the ketone compound

is selected from



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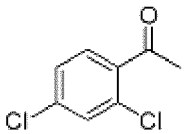
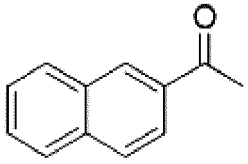


or



[0016] Further, the reaction system further includes a dissolution promoter, and the dissolution promoter is dimethyl sulfoxide or polyethylene glycol, and the polyethylene glycol is preferably PEG-400.

[0017] Further, the C1 to C8 alkyl is C1 to C8 linear alkyl, the C5 to C10 heteroaryl is a pyridine group, the alkoxy is C1 to C6 alkoxy, the heterocyclic radical in the C5 to C10 heterocyclic radical is piperidine, a substituent on the aryl in

the C5 to C10 aryl, the heteroaryl in the C5 to C10 heteroaryl, the carbocyclic radical in the C5 to C10 carbocyclic radical or the heterocyclic radical in the C5 to C10 heterocyclic radical is independently C1 to C6 linear alkyl or C1 to C6 alkoxy, and the amino donor is isopropylamine or D-alanine.

[0018] By means of the technical solutions of the present invention, an omega-transaminase of R-configuration having a high stereoselectivity, or a modified compound, functional equivalent, functional fragment or variant thereof may be used for highly efficient synthesis of a chiral amine of R-configuration with a relatively high chiral purity, and is therefore suitable for industrial production of chiral amines.

Brief description of the drawings

[0019] The accompanying drawings of the specification, which constitute a part of the application, are used for providing further understanding to the present invention. The exemplary embodiments of the present invention and illustration thereof are used for explaining the present invention, instead of constituting improper limitation to the present invention. In the accompanying drawings:

Fig. 1 shows a flowchart of a chemical reaction of a use of a transaminase derived from *Aspergillus terreus* and *Hyphomonas neptunium* in synthesis of a chiral amine according to an embodiment of the present invention;
Fig. 2 is an equation of a chemical reaction of a use of a transaminase derived from *Aspergillus terreus* and *Hyphomonas neptunium* in synthesis of a chiral amine according to an embodiment of the present invention;
Fig. 3 shows an identification result of enzyme digestion in the first embodiment of the present invention;
Fig. 4 shows a sequencing result of a mutant gene having been subjected to a Polymerase Chain Reaction (PCR) in the first embodiment of the present invention;
Fig. 5 shows an identification result of enzyme digestion in the fourth embodiment of the present invention; and
Fig. 6 shows a sequencing result of a mutant gene having been subjected to a PCR in the fourth embodiment of the present invention.

Detailed description of the embodiments

[0020] It needs to be noted that the embodiments in the application and the characteristics in the embodiments may be combined with each other if there is no conflict. The present invention will be expounded hereinafter with reference to the accompanying drawings and in conjunction with the embodiments.

Definition

[0021] The term "optional/random" or "optionally/randomly" means that an event or a situation in description thereinafter may happen or may not happen. The description includes that the event or the situation happens or does not happen. For example, "optionally substituted alkyl" refers to "unsubstituted alkyl" (alkyl that has not been substituted by a substituent) or "substituted alkyl" (alkyl that has been substituted by a substituent), as defined hereinafter.

[0022] The C1 to Cn used herein includes C1 to C2, C1 to C3, ...C1 to Cn. For example, the "C1 to C4" radical means that the part has 1 to 4 carbon atoms, that is, the radical includes 1 carbon atom, 2 carbon atoms, 3 carbon atoms or 4 carbon atoms.

[0023] The term "alkyl" used separately or in combination herein refers to optionally substituted linear chain or optionally substituted branched chain aliphatic hydrocarbons. The "alkyl" herein may preferably have 1 to about 20 carbon atoms, e.g. 1 to about 10 carbon atoms, 1 to about 8 carbon atoms, 1 to about 6 carbon atoms, 1 to about 4 carbon atoms or 1 to about 3 carbon atoms. The term "alkoxy" used separately or in combination herein refers to an alkyl ether group (O-alkyl). Nonrestrictive embodiments of alkoxy include: methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, tert-butoxy and so on.

[0024] The term "halogenated" or "halogen substituted" used separately or in combination herein means that one or more hydrogen atoms in an optionally substituted radical (such as alkyl, alkenyl and alkynyl) are replaced by atoms of fluorine, chlorine, bromine, iodine, or a combination thereof.

[0025] The term "aromatic base/aryl" used separately or in combination herein refers to optionally substituted aromatic hydrocarbyl having 6 to about 20 cyclization carbon atoms, e.g. 6 to about 12 or 6 to about 10 cyclization carbon atoms, and may be a condensed aromatic ring or a non-condensed aromatic ring.

[0026] The term "heteroaryl" used separately or in combination herein refers to optionally substituted univalent heteroaryl including 5 to about 20, e.g. 5 to about 12, or 5 to about 10 framework cyclization atoms, wherein one or more (e.g. 1 to 4, 1 to 3, or 1 to 2) cyclization atoms are heteroatoms, and the heteroatoms are independently selected from heteroatoms in oxygen, nitrogen, sulfur, phosphorus, silicon, selenium and tin, but are not limited thereby. A ring of the radical does not include two adjacent O or S atoms. Heteroaryl includes monocyclic heteroaryl or polycyclic heteroaryl

(such as dicyclic heteroaryl, tricyclic heteroaryl and so on).

[0027] The term "heterocycle" or "heterocyclic radical" used separately or in combination herein refers to a non-aromatic heterocycle, including heterocycloalkyl, and heterocycloalkenyl, wherein one or more (1 to 4, 1 to 3 or 1 to 2) cyclization atoms are heteroatoms, such as oxygen atoms, nitrogen atoms, or sulfur atoms. A heterocyclic radical may include a monoheterocyclic radical (a heterocyclic radical having one ring), or a polyheterocyclic radical (e.g. a diheterocyclic radical (a heterocyclic radical having two rings), a triheterocyclic radical and so on).

[0028] The term "carbocyclic radical" used separately or in combination herein refers to a non-aromatic carbon ring, including cycloalkyl and cycloalkenyl. The cycloalkyl may be monocyclic cycloalkyl or polycyclic cycloalkyl (e.g. having 2, 3, or 4 rings, such as dicyclic cycloalkyl), and may be a spiral ring or a bridge ring. A carbocyclic radical may have 3 to 20 carbon atoms, such as 3 to about 15 cyclization carbon atoms, 3 to about 10 cyclization carbon atoms or 3 to 6 cyclization carbon atoms, and may have 0, 1, 2 or 3 double bonds and/or 0, 1 or 2 triple bonds, e.g. cycloalkyl having 3 to 8 or 3 to 6 cyclization carbon atoms.

[0029] "Halogen" refers to fluorine, chlorine, bromine, and iodine, preferably fluorine, chlorine and bromine. Cyano refers to "-CN", hydroxyl refers to "-OH", mercapto refers to "-SH" and amino refers to "$-NH_2$".

[0030] The term "substituted" means that one or more hydrogen groups on a specific atom are substituted by a designated radical. If the normal valence of the designated atom is in, but is not beyond an existing condition, the substitution results in a stable compound.

[0031] As mentioned in the background, an omega-transaminase in the prior art still fails to satisfy demands for preparation of a compound of a chiral amine, and the present invention provides an omega-transaminase of R-configuration or a modified compound, functional equivalent, functional fragment or variant thereof in order to improve the situation above. The amino sequence of the omega-transaminase of R-configuration include a sequence selected from the following sequences: a) an amino acid sequence as shown in SEQ ID NO: 2 or 4; b) an amino acid sequence with at least 80% identity to the amino acid sequences as shown in SEQ ID NO: 2 or 4 and having the activity of an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the amino acid sequence is not the amino acid sequences encoded by a nucleotide sequences as shown in SEQ ID NO: 5 or 6; c) a protein which is derived from SEQ ID NO: 2 or 4 by subjecting the amino acid sequence as shown in SEQ ID NO: 2 or 4 to substitution, deletion or addition one or more amino acids, and having the activity of an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the amino acid sequence is not the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 2 or 4, wherein the high stereoselectivity refers to the content of one of the stereoisomers being at least about 1.1 times that of the other.

[0032] The omega-transaminase of R-configuration of the present invention refers to an omega-transaminase having a high R-configuration stereoselectivity. In an embodiment, the transaminase of the present invention refers to the transaminase as shown in SEQ ID NO: 2 or 4. The transaminase is a new transaminase obtained by subjecting transaminase genes taAT and taHN derived from *Aspergillus terreus* and *Hyphomonas neptunium* to mutation and modification by means of a molecular biological technique.

[0033] The amino acid sequence with at least 80% identity to the amino acid sequence as shown in SEQ ID NO: 2 or 4 and having the activity of an omega-transaminase with high stereoselective R-configuration catalytic activity refers to a sequence, which has at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 99.7% identity, for example, to the amino acid sequence as shown in SEQ ID NO: 2, but is not the amino acid sequence as shown in SEQ ID NO: 5. While keeping amino acids playing keyroles in the catalytic activity of the transaminase among the amino acid sequence as shown in SEQ ID NO: 2 or 4 unchanged, those skilled in the art may change remaining amino acid sequences of inactive sites, so that the amino acid sequence of an obtained transaminase has at least more than 80% identity to the amino acid sequence as shown in SEQ ID NO: 2, and the transaminase obtained in this way has the same transaminase activity as that of a transaminase having amino acid sequence as shown in SEQ ID NO: 2 or 4.

[0034] Similarly, one or more amino acids may be substituted, deleted or added to the amino acids in the amino acid sequence as shown in SEQ ID NO: 2 or 4 while keeping the amino acids playing key roles in the catalytic activity of the transaminase among the amino acid sequenceas shown in SEQ ID NO: 2 or 4 unchanged, thus a protein derived from SEQ ID NO: 2 or 4 can keep the high stereoselectivity of the transaminase as shown in SEQ ID NO: 2 or 4, wherein there may be one or more substituted, deleted or added bases, e.g. 1, 2, 3, 4, 5, 10, 20, 30 or 50 amino acids, e.g. substitution of conserved amino acids, wherein the amino acid sequence is not the amino acid sequence as shown in SEQ ID NO: 5. "Replacement of conserved amino acids" refers to combinations such as Gly, Ala; Val, lie, Leu; Asp, Glu; Asn, Gin; Ser, Thr; Lys, Arg; and Phe and Tyr.

[0035] The stereoselectivity means that when two stereisomers A and B are generated in a reaction, the yield of A is more than that of B, and the high stereoselectivity refers to the content of one of the stereoisomers being at least about 1.1 times that of the other, e.g. at least about 1.2 times, at least about 1.3 time, at least about 1.4 times, at least about 1.5 times, at least about 2 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 10 times, at least about 15 times, at least about 20 times, at least about 30 times, at least about 40 times, at least about 50 times, at least about 70 times, at least about 90 times, at least about 100 times or higher.

[0036] In the present invention, the modified compound of the omega-transaminase of R-configuration may be a chemical modified compound, such as a product of acylation, alkylation, or Polyethylene Glycolation (PEGylation), as long as these modified compounds maintain the activity of the omega-transaminase with the high stereoselective R-configuration catalytic activity. The functional equivalent refers to other peptide fragments that can realize the activity of the omega-transaminase of R-configuration. The functional fragment refers to a protein fragment that keeps the activity of the omega-transaminase with the high stereoselective R-configuration catalytic activity. The variant refers to a polypeptide derived from a parental protein by changing one or more amino acids at one or more (several) sites, i.e. by substation, insertion and/or deletion.

[0037] In a preferred embodiment of the present invention, the amino acid sequence of the transaminase is an amino acid sequence acquired by substituting leucine at the 38th site of the amino acid sequence as shown in SEQ ID NO: 2 by isoleucine. Such replacement between amino acids having similar properties enables the transaminase having the amino acid sequencereplaced maintains the activity of the transaminase having the amino acid sequence as shown in SEQ ID NO: 2 and high stereoselectivity.

[0038] The transaminase obtained by the present invention, which is an omega-transaminase with high stereoselective R-configuration catalytic activity, can be used for highly efficient synthesis of a chiral amine of R-configuration with a relatively high chiral purity, and is therefore suitable for industrial production of chiral amines. The present invention selects a splicing object and a splicing site in an optimized manner, so that a new transaminase variant obtained by transformation does not affect folding of proteins while maintaining the transaminase activity, having relatively high transaminase activity and high stereoselectivity.

[0039] A nucleotide is provided in another typical embodiment. The nucleotide encodes the omega-transaminase of R-configuration or the modified compound, functional equivalent, functional fragment or variant thereof, and an encoding rule of the nucleotide of the omega-transaminase of R-configuration or the modified compound, functional equivalent, functional fragment or variant thereof accords with a conventional codon usage table.

[0040] In a more preferred embodiment of the present invention, the sequences of the nucleotide include a sequence selected from one of the following sequences: a) a nucleotide sequence as shown in SEQ ID NO: 1 or 3; b) a nucleotide sequence which has at least 80% identity to the nucleotide sequence as shown in SEQ ID NO: 1 or 3 and encodes an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the nucleotide sequence is not the nucleotide sequence as shown in SEQ ID NO: 5 or 6; c) a nucleotide sequence with capable of hybridizing with the nucleotide sequence as shown in SEQ ID NO: 1 or 3 under highly stringent conditions and encoding an omega-transaminase having high stereoselective R-configuration catalytic activity, wherein the nucleotide sequence is not the nucleotide sequence as shown in SEQ ID NO: 4 or 6, wherein the high stereoselectivity refers to the content of one of the stereoisomers being at least about 1.1 times that of the other.

[0041] The nucleotide sequence with at least 80% identity to the nucleotide sequence as shown in SEQ ID NO.:1 or 3 and encoding the omega-transaminase having the high stereoselective R-configuration catalytic activity, having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.7%, 99.8% or 99.9% identity, for example, is not the nucleotide sequence as shown in SEQ ID NO: 5 or 6. While keeping nucleotides playing key roles in the catalytic activity of the transaminase unchanged based on the nucleotide sequence as shown in SEQ ID NO: 1 or 3, those skilled in the art may change remaining nucleotide sequence of inactive sites, so that the nucleotide sequence of an obtained transaminase has at least more than 80% identity to the nucleotide sequence as shown in SEQ ID NO: 1 or 3, and the transaminase obtained in this way has the same transaminase activity as that of a transaminase having nucleotide sequence as shown in SEQ ID NO: 1 or 3.

[0042] The nucleotide sequence capable of hybridizing with the nucleotide sequence as shown in SEQ ID NO: 1 or 3 under highly stringent conditions and encoding the omega-transaminase having the high stereoselective R-configuration catalytic activity is not the nucleotide sequence as shown in SEQ ID NO: 5 or 6. Similarly, a nucleotide sequencethat can be hybridized with the nucleotide sequence as shown in SEQ ID NO:1 or 3 under highly stringent conditions and encodes the omega-transaminase having the high stereoselective R-configuration catalytic activity is screened based on the nucleotide sequence as shown in SEQ ID NO: 1 or 3, and a variant sequence of the nucleotide sequence as shown in SEQ ID NO: 1 or 3, which is obtained in this way, have the same transaminase activity as that of the transaminase having the nucleotide sequence as shown in SEQ ID NO: 1 or 3.

[0043] The stereoselectivity means that when two stereisomers A and B are generated in a reaction, the yield of A is more than that of B, and the high stereoselectivity refers to the content of one of the stereoisomers being at least about 1.1 times that of the other, e.g. at least about 1.2 times, at least about 1.3 time, at least about 1.4 times, at least about 1.5 times, at least about 2 times, at least about 3 times, at least about 4 times, at least about 5 times, at least about 10 times, at least about 15 times, at least about 20 times, at least about 30 times, at least about 40 times, at least about 50 times, at least about 70 times, at least about 90 times, at least about 100 times or higher.

[0044] An exemplary highly stringent condition may be that the hybridization is performed at 65°C by using 6X SSC and a 0.5%SDS solution, and membrane washing is performed by 2X SSC, 0.1%SDS and 1X SSC, and 0.1%SDS once respectively.

[0045] The term "identity" used in the present invention has a meaning generally known in the art. Those skilled in the art are also familiar with rules and standards for measuring the identity between different sequences. Sequences limited by the present invention with identities of different degrees also need to have the activity of the omega-transaminase with the high stereoselectivity R-configuration catalytic activity at the same time. Those skilled in the art generally know methods and means for screening the variant sequences by using the activity of the omega-transaminase with the high stereoselective R-configuration catalytic activity, and may be taught by the content disclosed by the application to acquire such variant sequences easily.

[0046] It is known by those skilled in the art that a qualifier used for limiting the amino acid sequences or polynucleotides is "include", but it does not mean that other sequences unrelated to functions of the amino acid sequences or polynucleotides may be added randomly to two ends of the amino acid sequences or polynucleotides. It is known by those skilled in the art that it is necessary to add proper restriction sites of restriction endonuclease, or additional initiator codons or stop codons and so on to both ends of the polynucleotides so as to meet requirements of a recombination operation. Therefore, these situations cannot be truly covered if the sequences are limited by closed expression.

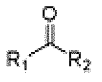
[0047] It is generally known by those skilled in the art that one or more codons in the nucleotide sequences may be replaced equivalently without changing the encoded amino acids. For example, the leucine Leu encoded by CTT is replaced by CTA, CTC or CTG, and the number of replaced codons may be one or more, e.g. 1, 2, 3, 4, 5, 6, 8, 9, 10, 15, 20, 30, 40 or 50, and a codon usage table is generally known in the art.

[0048] A recombinant vector is provided according to another aspect of the present invention. Any foregoing nucleotide is effectively connected in the recombinant vector. The recombinant vector of the present invention includes, but is not limited to a recombinant expression vector, and may also include a recombinant cloning vector. The recombinant vector may be a prokaryotic expression vector or a eukaryotic expression vector. In an embodiment of the present invention, the recombinant vector is a recombinant prokaryotic expression vector that can induce expression, e.g. a pET series vector that induces gene expression by IPTG, such as a pET22b vector. In the present invention, recombinant vectors having the nucleotide sequence as shown in SEQ ID NO: 1 and SEQ ID NO: 3 are pET22b-CM32 and pET22b-CM33, wherein the "effectively connected" refers to such a connection method that a polynucleotide is placed at a proper location of the vector so that the polynucleotide is copied, transcribed and/or translated correctly and smoothly.

[0049] A host cell is provided according to another aspect of the present invention. Any foregoing recombinant vector is transformed or transfected into the host cell. The host cell of the present invention includes a prokaryotic host cell and a eukaryotic host cell. In an embodiment of the present invention, the host cell is a prokaryotic host cell, such as *Escherichia Coli,* preferably, DH5α (DE3).

[0050] A method for synthesizing a chiral amine is provided according to another aspect of the present invention. The method includes the following steps: making a ketone compound, any omega-transaminase of R-configuration or the modified compound, functional equivalent, functional fragment or variant thereof, pyridoxal phosphate, and an amino donor to react in a reaction system so as to obtain the chiral amine. The method for synthesizing a chiral amine according to the present invention only needs to utilize the transaminase of the present invention and make appropriate adjustment to parameters including the components, proportions, use amounts, pH values, temperature and reaction time and so on of reaction raw materials of the reaction system based on a conventional method for preparing a chiral compound through a reaction catalyzed by a biological enzyme in the art.

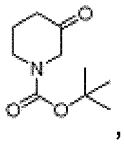
[0051] In a preferred embodiment of the present invention, the ketone compound is



,

where R_1 and R_2 are independently C_1 to C_8 alkyl, C_5 to C_{10} cycloalkyl, C_5 to C_{10} aryl or C_5 to C_{10} heteroaryl; or R_1 and R_2 form a C_5 to C_{10} heterocyclic radical, a C_5 to C_{10} carbocyclic radical or C_5 to C_{10} heteroaryl with a carbon on a carbonyl group; heteroatoms in the C_5 to C_{10} heterocyclic radical and C_5 to C_{10} heteroaryl are independently selected from at least one of nitrogen, oxygen and sulfur; the aryl in the C_5 to C_{10} aryl, the heteroaryl in the C_5 to C_{10} heteroaryl, the carbocyclic radical in the C_5 to C_{10} carbocyclic radical or the heterocyclic radical in the C_5 to C_{10} heterocyclic radical is independently unsubstituted or is substituted by at least one radical of halogen, alkoxy or alkyl; preferably, the ketone compound

is selected from



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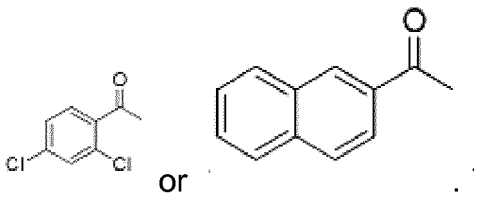

or .

The ketone compound is a commercial raw material or a raw material that can be prepared easily and is cheap, and can therefore satisfy demands of mass production.

[0052] In another preferred embodiment of the present invention, the reaction system further includes a dissolution promoter, and the dissolution promoter is dimethyl sulfoxide or polyethylene glycol, and the polyethylene glycol is preferably PEG-400. The dissolution promoter can better dissolve the raw materials above so that the reaction can be carried out, and PEG-400 has the best dissolution promoting effect.

[0053] In another preferred embodiment of the present invention, the C1 to C8 alkyl is C1 to C8 linear alkyl, the C5 to C10 heteroaryl is a pyridine group, the alkoxy is C1 to C6 alkoxy, the heterocyclic radical in the C5 to C10 heterocyclic radical is piperidine, a substituent on the aryl in the C5 to C10 aryl, the heteroaryl in the C5 to C10 heteroaryl, the carbocyclic radical in the C5 to C10 carbocyclic radical or the heterocyclic radical in the C5 to C10 heterocyclic radical is independently C1 to C6 linear alkyl or C1 to C6 alkoxy, and the amino donor is isopropylamine or D-alanine. The raw materials above are commercial raw materials or raw materials that can be prepared easily and are cheap, and can therefore satisfy demands of large-scaled production.

[0054] In a preferred embodiment of the present invention, the reaction system contains a buffer solution for maintaining the pH value of the reaction system in a range of 7.0 to 9.5, and/or wherein the ratio of the use amount of the ketone compound to that of the dissolution promoter is 1g/1mL to 15mL; and/or wherein the ratio of the use amount of the ketone compound to that of the buffer solution is 1 g/15mL to 50mL, and/or wherein the ratio of the use amount of the ketone compound to that of pyridoxal phosphate is 1g/0.01g to 0.1 g, and/or wherein the ratio of the use amount of the ketone compound to that of the amino donor is 1eq/1eq to 5eq, and/or wherein the ratio of the use amount of the ketone compound to that of the omega-transaminase of R-configuration is 1g/0.2g to 10g; and/or wherein the temperature of the reaction system is 20°C to 45°C and the reaction time is 12h to 48h, and/or wherein the buffer solution is a phosphate buffer solution or a triethanolamine having a pH value of pH=9.3 to 9.5.

[0055] In another preferred embodiment of the present invention, the method further includes a step that the reaction system is regulated to pH≥10 by an alkaline, and a product chiral amine in an aqueous phase is extracted by an organic solvent. Preferably, the alkaline is sodium hydroxide or potassium hydroxide, and the organic solvent is ethyl acetate, methyl tert-butyl ether or 2-methyltetrahydrofuran.

[0056] An R-configuration chiral amine is provided in another typical embodiment of the present invention. The R-configuration chiral amine is synthesized by using any method above. The R-configuration chiral amine prepared by the transaminase of the present invention has a high chiral purity which may be as high as more than 98%.

[0057] The beneficial effect of the present invention will be described below in combination with specific embodiments.

[0058] The methods are conventional methods unless otherwise specified in the following embodiments and the used experimental materials may be obtained easily from commercial corporations unless otherwise specified.

Embodiment 1: Preparation of transaminase AH-TACM33 derived from *Aspergillus terreus* and *Hyphomonas neptunium*

[0059] Specific steps of a preparation method of a transaminase AH-TACM33 of the present invention are as follows:

(1) Template construction

[0060] Sangon Biotech (Shanghai) Co., Ltd. is entrusted to carry out whole gene synthesis of transaminase genes taAT *(Aspergillus terreus)* (the nucleotide sequence is the gene sequenceas shown in SEQ ID NO: 5 in the sequencing list, and the amino acid sequence is as shown in SEQ ID NO: 23) and taHN *(Hyphomonas neptunium)* (the nucleotide sequence is the gene sequence as shown in SEQ ID NO: 6 in the sequencing list, and the amino acid sequenceis as shown in SEQ ID NO: 24) derived from *Aspergillus terreus* and *Hyphomonas neptunium.* The synthesized genes taAT and taHN are connected to a vector pUC57 respectively to obtain recombinant plasmids pUC57-taAT and pUC57-taHN, then the recombinant plasmids pUC57-taAT and pUC57-taHN are subjected to enzyme digestion simultaneously by using restriction endonuclease Nde I and Xho I, and purified recovered fragments taAT and taHN are obtained through gel recovery and used as templates of PCR in the next step.

(2) Primer design

[0061] Specific primers designed according to the transaminase gene derived from *Aspergillus terreus* are as follows:

taAT A: 5'-CCGCTCGAGGTTACGCTCGTTGTAGTCAATTTC-3' (SEQ ID NO: 7)
taAT S: 5'-GGAATTCCATATGGCGTCTATGGACAAAG-3' (SEQ ID NO: 8)

[0062] Specific primers designed according to the transaminase gene derived from *Hyphomonas neptunium* are as follows:

taHN A: 5'-CCGCTCGAGCGGTGCATAGGTTACCGGTTC-3' (SEQ ID NO: 9)
taHN S: 5'-GGAATTCCATATGCTGACCTTCCAAAAAGTACTGAC-3' (SEQ ID NO: 10)

[0063] In the meanwhile, 6 pairs of primers are designed according to different sites, which are respectively as follows:

CM31A: 5'-GAACTTCAGACCGCGGGTGACAATCAG-3' (SEQ ID NO: 11)
CM31S: 5'-CACCCGCGGTCTGAAGTTCCTGC-3' (SEQ ID NO: 12)
CM32A: 5'-CGGCGGAACACGACGAACGGTACG-3' (SEQ ID NO: 13)
CM32S: 5'-TTCGTCGTACTCCGCCGGGCGCAC-3' (SEQ ID NO: 14)
CM33A: 5'-TAGCCTGCGCCCTCGGTCAGGTGAG-3' (SEQ ID NO: 15)
CM33S: 5'-GACCGAGGGCGCAGGCTACAATATC-3' (SEQ ID NO: 16)
CM34A: 5'-CCCTTCAGACCACGCGTAACGATGATC-3' (SEQ ID NO: 17)
CM34S: 5'-TTACGCGTGGTCTGAAGGGTGTGCGTG-3' (SEQ ID NO: 18)
CM35A: 5'-CCAGGCGGAGTACGACGTACAGTACGAG-3' (SEQ ID NO: 19)
CM35S: 5'-TACGTCGTGTTCCGCCTGGCGCAATC-3' (SEQ ID NO: 20)
CM36A: 5'-GCCGCTGCCTTCCGTCGCGTTACC-3' (SEQ ID NO: 21)
CM36S: 5'-GACGGAAGGCAGCGGCTTCAACATC-3' (SEQ ID NO: 22)

(3) Acquisition of a new transaminase

[0064] The specific primer taAT S (a forward primer) designed on the transaminase gene derived from *Aspergillus terreus* is combined with any reverse primer in 3 reverse primers (CM31A, CM32A, CM33A) among the above 6 pairs of primers to amplify a fragment of the transaminase gene derived from *Aspergillus terreus,* or taHN A (a reverse primer) is combined with any one of 3 forward primers (CM36S, CM35S and CM34S) among the 6 pairs of primers to amplify a fragment of the transaminase gene derived from *Hyphomonas neptunium.* Subsequently, the two fragments of different origins, which are obtained from the amplification, are integrated so as to obtain a transformed transaminase gene.

[0065] Similarly, the specific primer taAT A (a reverse primer) designed on the transaminase gene derived from *Aspergillus terreus* is combined with any forward primer in 3 forward primers (CM33S, CM32S, CM31S) among the 6 pairs of primers to amplify a fragment of the transaminase gene derived from *Aspergillus terreus,* or taHN S (a forward primer) is combined with any one of 3 reverse primers (CM34A, CM35A and CM36A) among the 6 pairs of primers to amplify a fragment of the transaminase gene derived from *Hyphomonas neptunium.* Subsequently, the two fragments of different origins, which are obtained from the amplification, are integrated so as to obtain a transformed transaminase

gene.

[0066] Specific transformation steps are expounded by taking a transaminase obtained by integrating the fragment acquired by amplifying the forward primer taAT S and the reverse primer CM33A and the fragment obtained by amplifying the reverse primer taHN A and the forward primer CM33S, and a transaminase obtained by integrating the fragment acquired by amplifying the forward promer taAT S and the reverse primer CM32A and the fragment acquired by amplifying the reverse primer taHN A and the forward primer CM32S as examples.

[0067] Steps for obtaining the transaminase AH-TACM33 are as follows:

Step 1: Acquisition of fragment A: the recovered fragment taAT is used as a PCR template, taAT S and CM33A are used as primers to perform PCR amplification and a product is recovered by gel extraction and purification to acquire a fragment A.

Step 2: Acquisition of fragment B: the recovered fragment taHN is used as a PCR template, taHN A and CM33S are used as primers to perform PCR amplification and a product is recovered by gel extraction and purification to acquire a fragment B.

Step 3: Acquisition of fragment CM33: the acquired fragment A and fragment B are used as templates and primers for each other, PCR amplification is performed for 5 cycles, then the primers taAT S and taHN A are added into the PCR system directly, overlapped PCR amplification is performed, and a product is recovered by gel extraction and purification to acquire a fragment CM33.

PCR system: fragment A 1μL, fragment B 11μL, PCR MIX 5μL, ddH_2O 4.5p1.

PCR procedure: 95°C 3min; (95°C 30s, 57°C 30s, 72°C 90s, 5 cycles); 72°C 1 min.

0.2μL of the primer taAT S and 0.2μL of the primer taHN A are added into the system respectively.

PCR procedure: 95°C3 min; (95°C 30s, 57°C 30s, 72°C 90s, 30 cycles); 72°C 10min.

Step 4: Acquisition of recombinant plasmid pET22b-CM33: the fragments CM33 and pET-22b (+) are subjected to enzyme digestion simultaneously by using Nde I and Xho I, ligation is performed by using a T4 DNA ligase, and a ligation product is transformed into a competent cell of an DH5α strain of *Escherichia coli,* resuscitated by a shaker, and then coated in an LB culture dish containing ampicillin having a final concentration of 50μg/ml, and cultured overnight in an incubator at 37°C. A single colony on the culture dish is selected and inoculated in an LB liquid culture medium containing ampicillin having a final concentration of 50μg/ml, and subjected to shake culture at 180r/min and 37°C overnight. A plasmid is extracted, subjected to PCR and identified by enzyme digestion, and an identification result of the enzyme digestion is as shown in Fig. 3.

Fig. 3 shows an identification diagram of the plasmid pET22b-CM33 having subjected to double digestion of the enzyme Nde I and the enzyme Xho I, wherein 1 represents an empty vector pET22b; 2 represents markers of the sizes of DNA molecules (10000bp, 8000bp, 6000bp, 5000bp, 4000bp, 3500bp, 3000bp, 2500bp, 2000bp, 1500bp, 1000bp, 750bp, 500bp, 250bp respectively from top to bottom) and 3 represents pET22b-CM33-DH5α. It may be seen from Fig. 3 that a relatively weak band having a fragment size of about 1000bp after the enzyme digestion is a target fragment (a plasmid band having the target fragment removed is relatively strong), thus it may be determined that the insertion direction and size of an insertion sequence of the recombinant plasmid pET22b-CM33 are correct.

Step 5: Acquisition of BL21/pET22b-CM33: the obtained recombinant plasmid pET22b-CM33 is directly transformed into *Escherichia coli* BL21 (DE3), resuscitated by a shaker, and then coated in an LB culture dish containing ampicillin having a final concentration of 50μg/ml, and cultured at 37°C overnight; a single colony in the culture dish is selected and inoculated in 5ml of an LB liquid culture medium containing ampicillin having a final concentration of 50μg/ml, and cultured at 180r/min and 37°C overnight; bacterial liquid is sent to Sangon Biotech (Shanghai) Co., Ltd. to be sequenced, and after being verified correctly through gene sequencing, the plasmid is named BL21/pET22b-CM33. The sequencing result is shown in Fig. 4 and it may be seen from Fig. 4 that the gene sequence carried by the BL21/pET22b-CM33 plasmid in the sequencing result is completely as expected and there is no mutated base. After being identified correctly by the sequencing, the recombinant plasmid is a target plasmid sequence.

Step 6: Preparation of transaminase AH-TACM33: a bacterial liquid of the BL21/pET22b-CM33 is transplanted in an LB liquid culture medium containing ampicillin having a final concentration of 50μg/ml, subjected to shake culture at 180r/min and 37°C, and when the OD600 value is 0.6 to 0.8, IPTG is added until the final concentration is 0.2mM, and the culture solution is transposed at 25°C to induce expression; after the induction is performed for 16h, the bacterial liquid is taken out and centrifuged at 12000r/min for 10min, and thalli are collected; after cells are disrupted, the thalli are centrifuged at 4°C and 12000r/min for 20min to obtain a supernatant which is a prepared transaminase AH-TACM33 having an amino acid sequence as shown in SEQ ID NO: 4 and a corresponding nucleotide sequence as shown in SEQ ID NO: 3.

Embodiment 2: Activity experiment 1 of transaminase AH-TACM33

[0068] 1g of a major raw material (N-Boc-3-piperidone, CAS: 79099-07-3) and 1 mL of dimethyl sulfoxide are added into a reaction flask. After the raw materials are dispersed, 50 mL of a triethanolamine buffer solution having a concentration of 0.2mol/L and a pH value regulated to 9.3 to 9.5 by concentrated hydrochloric acid in an ice bath, 0.765g of isopropylamine, 0.01g of pyridoxal phosphate, and 0.01 g of the transaminase AH-TACM33 are added, and stirred for 12h at a constant temperature of 30°C, wherein the pH value of the system is 9.5. The pH value of the system is regulated to above 10 by 2N NaOH, extraction is performed twice by ethyl acetate, and an organic phase is dried, filtered and concentrated to obtain a crude product (English name: (R)-1-N-Boc-3-aminopiperidine, CAS: 188111-79-7). It is detected by Gas Chromatography (GC) that the conversion rate is 90% and the e.e value is 100%.

[0069] Nuclear Magnetic Resonance (NMR) data of the obtained product is as follows: 1 H-NMR (300MHz, CDCl3) δ 4.00-3.78 (m, 2H), 3.80 (m, 2H), 3.60 (m, 1 H), 1.90 (m, 1 H). 1.70 (m, 1 H), 1.60-1.40 (m, 12H), 1.30 (m, 1 H) ppm.

Embodiment 3: Activity experiment 2 of transaminase AH-TACM33

[0070] 0.1g of a major raw material (2,4-dichloroacetophenone, CAS:2234-16-4) and 1.5 mL of polyethylene glycol PEG-400 are added into a reaction flask. After the raw materials are dispersed, 23.5 mL of a phosphate buffer solution (pH8.0), 0.031g of isopropylamine, 0.0075g of pyridoxal phosphate, and 0.02g of the transaminase AH-TACM33 are added, and stirred for 20h at a constant temperature of 45°C, wherein the pH value of the system is 8.0. The pH value of the system is regulated to above 10 by 2N NaOH, extraction is performed twice by ethyl acetate, and an organic phase is dried, filtered and concentrated to obtain a crude product ([(R)-(+)-1-(2,4-dichlorophenyl)ethyl]amine). It is detected by GC that the conversion rate is 82% and the e.e value is 100%.

NMR data of the obtained product is as follows: 1 H NMR (400MHz, DMSO D6):δ=7.67 (d 1H), 7.60 (d, 1H), 7.47 (dd, 1H), 7.34 (dd, 4H), 7.23-7.12 (m, 6H), 4.84 (s, 1 H), 4.47 (quartet, 1 H), 1.31 (d, 3H).

Embodiment 4: Preparation of transaminase AH-TACM32 derived from *Aspergillus terreus* and *Hyphomonas neptunium*

[0071] Specific steps of a preparation method of a transaminase AH-TACM32 of the present invention are as follows:

(1) Template construction

[0072] Recombinant plasmids pUC57-taAT and pUC57-taHN are obtained according to the method of Embodiment 1. The recombinant plasmids pUC57-taAT and pUC57-taHN are subjected to enzyme digestion simultaneously by using restriction endonuclease Nde I and Xho I, and purified recovered fragments taAT and taHN are obtained through gel recovery and used as templates of PCR in the next step.

(2) Primer design

[0073] Primer design is the same as Embodiment 1.

(3) Acquisition of a new transaminase

[0074] Step 1: Acquisition of fragment E: the recovered fragment taAT is used as a PCR template, taAT S and CM32A are used as primers to perform PCR amplification and a product is recovered by gel extraction and purification to acquire a fragment E.

[0075] Step 2: Acquisition of fragment F: the recovered fragment taHN is used as a PCR template, taHN A and CM32S are used as primers to perform PCR amplification and a product is recovered by gel extraction and purification to acquire a fragment F.

[0076] Step 3: Acquisition of fragment CM32: the acquired fragment E and fragment F are used as templates and primers for each other, PCR amplification is performed for 5 cycles, then the primers taAT S and taHN A are added into the PCR system directly, overlapped PCR amplification is performed, and a product is recovered by gel extraction and purification to acquire a fragment CM32.

[0077] Step 4: Acquisition of recombinant plasmid pET22b-CM32: the fragments CM32 and pET-22b (+) are subjected to enzyme digestion simultaneously by using Nde I and Xho I, ligation is performed by using a T4 DNA ligase, and a ligation product is transformed into a competent cell of an DH5α strain of *Escherichia coli,* resuscitated by a shaker, and then coated in an LB culture dish containing ampicillin having a final concentration of 50μg/ml, and cultured overnight in an incubator at 37°C. A single colony on the culture dish is selected and inoculated in an LB liquid culture medium

containing ampicillin having a final concentration of 50μg/ml, and subjected to shake culture at 180r/min and 37°C overnight. A plasmid is extracted, subjected to PCR and identified by enzyme digestion, and an identification result of the enzyme digestion is as shown in Fig. 5.

[0078] Fig. 5 shows an identification diagram of the plasmid pET22b-CM32 having subjected to double digestion of the enzyme Nde I and the enzyme Xho I, wherein 1 represents markers of the sizes of DNA molecules (10000bp, 8000bp, 6000bp, 5000bp, 4000bp, 3500bp, 3000bp, 2500bp, 2000bp, 1500bp, 1000bp, 750bp, 500bp, 250bp from top to bottom), 2 represents pET22b-CM32-DH5α and 3 represents an empty vector pET22b.

[0079] It may be seen from Fig. 5 that a relatively weak band having a fragment size of about 1000bp after the enzyme digestion is a target fragment (a plasmid band having the target fragment removed is relatively strong), thus it may be determined that the insertion direction and size of an insertion sequence of the recombinant plasmid pET22b-CM32 are correct, so as to obtain the recombinant plasmid pET22b-CM32.

[0080] Step 5: Acquisition of BL21/pET22b-CM32: the obtained recombinant plasmid pET22b-CM32 is directly transformed into *Escherichia coli* BL21 (DE3), resuscitated by a shaker, and then coated in an LB culture dish containing ampicillin having a final concentration of 50μg/ml, and cultured at 37°Covernight; a single colony in the culture dish is selected and inoculated in 5ml of an LB liquid culture medium containing ampicillin having a final concentration of 50μg/ml, and cultured at 180r/min and 37°C overnight; bacterial liquid is sent to Sangon Biotech (Shanghai) Co., Ltd. to be sequenced, and after being verified correctly through gene sequencing, the plasmid is named BL21/pET22b-CM32.

[0081] The sequencing result is shown in Fig. 6 and it may be seen from Fig. 6 that the gene sequence carried by the BL21/pET22b-CM32 plasmid in the sequencing result is completely as expected and there is no mutated base. After being identified correctly by the sequencing, the recombinant plasmid is a target plasmid sequence.

[0082] Step 6: Preparation of transaminase AH-TACM32: a bacterial liquid of the BL21/pET22b-CM32 is transplanted in an LB liquid culture medium containing ampicillin having a final concentration of 50μg/ml, subjected to shake culture at 180r/min and 37°C, and when the OD600 value is 0.6 to 0.8, IPTG is added until the final concentration is 0.2mM, and the culture solution is transposed at 25°C to induce expression; after the induction is performed for 16h, the bacterial liquid is taken out and centrifuged at 12000r/min for 10min, and thalli are collected; after cells are disrupted, the thalli are centrifuged at 4°C and 12000r/min for 20min to obtain a supernatant which is a prepared transaminase AH-TACM32 having an amino acid sequence as shown in SEQ ID NO: 2 and the corresponding nucleotide sequence as shown in SEQ ID NO: 1.

Embodiment 5: Activity experiment of transaminase AH-TACM32

[0083] 0.1 g of a major raw material (2-acetonaphthone, CAS: 93-08-3) and 1 mL of polyethylene glycol PEG-400 are added into a reaction flask. After the raw materials are dispersed, 24 mL of a phosphate buffer solution (pH7.0), 0.17g of isopropylamine, 0.01g of pyridoxal phosphate, and 0.004g of the transaminase AH-TACM32 are added, and stirred for 48h at a constant temperature of 20°C, wherein the pH value of the system is 7.0. The pH value of the system is regulated to above 10 by 2N NaOH, extraction is performed twice by ethyl acetate, and an organic phase is dried, filtered and concentrated to obtain a crude product. It is detected by GC that the conversion rate is 20% and the e.e value is 100%.

[0084] NMR data of the obtained product is as follows: 1 H NMR (400MHz, CDCl3) δ 7.86-7.76 (m, 4H), 7.52-7.41 (m, 3H), 4.29 (q, J=6.4Hz, 1 H), 1.74 (br s, 2H), 1.48 (d, J=6.4Hz, 3H).

[0085] The present invention further verifies the transaminase activity of the transaminase AH-TACM33 by using the major raw material (2-acetonaphthone, CAS: 93-08-3) and verifies the transaminase activity of the transaminase AH-TACM32 by using a major raw material (N-Boc-3-piperidone, CAS: 79099-07-3) and a major raw material (2,4-dichloroacetophenone, CAS:2234-16-4), specific method steps are the same as the embodiments above.

Embodiment 6

[0086] Based on the transaminase having an amino acid sequence as shown in SEQ ID NO: 2, the leucine at the 38th site of the transaminase is subjected to site-directed mutagenesis to be replaced with isoleucine, so as to obtain a transaminase having an sequence as shown in SEQ ID NO: 25.

[0087] An enzyme activity experiment is carried out to detect the transaminase, and detection steps are as follows:

a bacterial liquid of mutants is transplanted to 100ml of an LB liquid culture medium containing ampicillin having a final concentration of 50μg/ml, subjected to shake culture at 180r/min and 37°C, and when the OD600 value is 0.6 to 0.8, IPTG is added until the final concentration is 0.2mM, and the culture solution is transposed at 25°C to induce expression. In the meanwhile, an IPTG inducer-free culture solution is set as a negative control. After the induction is performed for 16h, the bacterial liquid is taken out and centrifuged at 12000r/min for 5min, and thalli are collected. 0.5g of bacterial sludge is weighed andre-suspended in 2.5 mL of a phosphate buffer solution (pH8.0) and cells of the thalli are disrupted by an ultrasonic disruptor. Ultrasonic parameters include: probe diameter 6mm, power 200W,

working time 2s, and interval 6s, totally 10min. After the ultrasonic processing, centrifugation is performed at 12000r/min for 20min at 4°C to obtain an ultrasonic supernatant and precipitate, and the supernatant is inputted in a reaction to verify transaminase activity.

[0088] 0.1 g of a major raw material (acetophenone, CAS: 98-86-2) is added to a reaction flask, the raw material is dispersed in 13.5mL of a phosphate buffer solution having a concentration of 0.1M (pH8.0), 0.356g of D-alanine, 0.002g of β-NAD+, 0.0192g of lactic dehydrogenase, 0.006g of glucose dehydrogenase, 0.432g of glucose, 0.004g of pyridoxal phosphate, and 2.5mL of an omega-transaminase of R-configuration having a sequence encoded by SEQ ID NO: 25, the pH value of the system is 7.0, stirring is performed at a constant temperature of 30°C for 16h, the pH value of the system is regulated to above 10 by NaOH having a concentration of 2N, extraction is performed twice by ethyl acetate, and an organic phase is dried, filtered and concentrated to obtain a crude product (English name: (R)-1-phenethylamine, CAS: 3886-69-9). It is detected by GC that the conversion rate is 83% and the e.e value is 99.5%.

[0089] NMR data of the obtained product is as follows: 1 H NMR(CDCl3, 400MHz, 300K) δ (ppm): 7.36-7.29 (m, 4H), 7.26-7.19 (m, 1 H), 4.11 (q, J=6.6Hz, 1 H), 1.53 (bs, 2H), 1.38 (d, J=6.6Hz, 3H).

[0090] The result shows that the transaminase in the above embodiments of the present invention may achieve similar yields and enantiomer purity, thereby obtaining corresponding chiral amines of R-configuration.

[0091] It may be seen from the foregoing description that the embodiments of the present invention have achieved the following technical effect: a novel transaminase disclosed by the present invention catalyzes the transfer of an amino in an amino donor to prochiral ketones or aldehydes, thereby generating corresponding chiral amines of R-configuration. A target product having a high purity may be obtained by utilizing a synthesis method of the novel transaminase of the present invention, and the optical purity of the obtained product is stabilized at above 98%. The synthesis method, which is simple, applies easily available raw materials and has mild chemical reaction conditions, high yield and high enantiomer purity, and simple operations in the whole production process, is a feasible synthesis process with little pollution and provides a new approach and method for preparation of chiral amines.

[0092] The above are only preferred embodiments of the present invention, but are not used for limiting the present invention. For those skilled in the art, the present invention may have various modifications and changes. Any modifications, equivalent replacements, improvements and the like made within the spirit and principles of the present invention shall be included in the scope of protection of the present invention.

Sequencing List

<110> ASYMCHEM LABORATORIES (TIANJIN) CO.,LTD
ASYMCHEM LIFE SCIENCE (TIANJIN) CO.,LTD
TIANJIN ASYMCHEM PHARMACEUTICAL CO.,LTD
ASYMCHEM LABORATORIES (FUXIN) CO.,LTD
JILIN ASYMCHEM LABORATORIES CO.,LTD

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and Neptune silk Aeromonas

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atcctggttg aaatggttgc gaaatccggc attcgcgacg cgtttgtcga actgattgtc 360

acccgcggtc tgaagggtgt gcgtggcacc cgtccggaag acattgtaaa caacctgtac 420

atgttcgtgc agccgtacgt ctgggttatg gaaccggaca tgcagcgtgt tggcggcagc 480

gcagttgtag cccgtaccgt tcgtcgtact ccgccgggcg cactggatcc gactattaaa 540

aacctgcagt ggggcgatct ggttcgcggt ctgatggaag caggcgaccg tgattctttc 600

tttcctattc tgccggatgg cgacggtaac gcgacggaag gcgcaggcta caatatcgta 660

ctggtgcgta acggcgagct gcataccccg cgtcgtggcg ttctggaagg tattacccgt 720

cgtacggtgc tggagattgc agctgctcgc ggcctgaaaa ctcacgtcac cgaaatccca 780

atccaggccc tgtatgaatg cgacgaactg tttatgtgca gcaccgcggg cggtatcatg 840

ccactggtgc tgctggatgg caacattgta ggtgacggca ccgttggtcc ggtcacccgc 900

atgatttggg aagcgtactg ggacctgcac gacgacccgc agctgtctga accggtaacc 960

tatgcaccgc tcgag 975

<210> SEQ ID NO.2
<211> 325
<212> PRT
<213> Artificial design

<220>
<223> recombinant transaminase AH-TACM32 derived from Aspergillus terreus and Neptune silk Aeromonas

<400> 2

Met Ala Ser Met Asp Lys Val Phe Ala Gly Tyr Ala Ala Arg Gln Ala
1 5 10 15

Ile Leu Glu Ser Thr Glu Thr Thr Asn Pro Phe Ala Lys Gly Ile Ala
20 25 30

Trp Val Glu Gly Glu Leu Val Pro Leu Ala Glu Ala Arg Ile Pro Leu
35 40 45

Leu Asp Gln Gly Phe Met His Ser Asp Leu Thr Tyr Asp Val Pro Ser
50 55 60

Val Trp Asp Gly Arg Phe Phe Arg Leu Asp Asp His Ile Thr Arg Leu
65 70 75 80

Glu Ala Ser Cys Thr Lys Leu Arg Leu Arg Leu Pro Leu Pro Arg Asp
85 90 95

Gln Val Lys Gln Ile Leu Val Glu Met Val Ala Lys Ser Gly Ile Arg
100 105 110

Asp Ala Phe Val Glu Leu Ile Val Thr Arg Gly Leu Lys Gly Val Arg
115 120 125

Gly Thr Arg Pro Glu Asp Ile Val Asn Asn Leu Tyr Met Phe Val Gln
130 135 140

Pro Tyr Val Trp Val Met Glu Pro Asp Met Gln Arg Val Gly Gly Ser
145 150 155 160

Ala Val Val Ala Arg Thr Val Arg Arg Thr Pro Pro Gly Ala Leu Asp
165 170 175

Pro Thr Ile Lys Asn Leu Gln Trp Gly Asp Leu Val Arg Gly Leu Met
180 185 190

Glu Ala Gly Asp Arg Asp Ser Phe Phe Pro Ile Leu Pro Asp Gly Asp
195 200 205

Gly Asn Ala Thr Glu Gly Ala Gly Tyr Asn Ile Val Leu Val Arg Asn
210 215 220

Gly Glu Leu His Thr Pro Arg Arg Gly Val Leu Glu Gly Ile Thr Arg
225 230 235 240

Arg Thr Val Leu Glu Ile Ala Ala Ala Arg Gly Leu Lys Thr His Val
245 250 255

Thr Glu Ile Pro Ile Gln Ala Leu Tyr Glu Cys Asp Glu Leu Phe Met
260 265 270

Cys Ser Thr Ala Gly Gly Ile Met Pro Leu Val Leu Leu Asp Gly Asn
275 280 285

Ile Val Gly Asp Gly Thr Val Gly Pro Val Thr Arg Met Ile Trp Glu
290 295 300

Ala Tyr Trp Asp Leu His Asp Asp Pro Gln Leu Ser Glu Pro Val Thr
305 310 315 320

Tyr Ala Pro Leu Glu
325

<210> SEQ ID NO.3
<211> 975
<212> DNA
<213> Artificial design

<220>
<223> recombinant transaminase gene AH-TACM33 derived from Aspergillus terreus and Neptune silk Aeromonas

<400> 3
atggcgtcta tggacaaagt ttttgcaggt tacgcggccc gtcaagcgat cctggaatct 60

actgagacta ccaacccgtt cgcgaaaggt atcgcgtggg tagaaggtga actggttccg 120

ctggcggaag cacgcatccc gctgctggat cagggcttca tgcattccga cctgacgtat 180

gacgtgccgt ctgtgtggga tggccgcttc ttccgtctgg atgatcacat cactcgtctg 240

gaagcatcct gcactaaact gcgtctgcgc ctgcctctgc cgcgtgatca ggtgaaacag 300

atcctggttg aaatggttgc gaaatccggc attcgcgacg cgtttgtcga actgattgtc 360

acccgcggtc tgaagggtgt gcgtggcacc cgtccggaag acattgtaaa caacctgtac 420

atgttcgtgc agccgtacgt ctgggttatg gaaccggaca tgcagcgtgt tggcggcagc 480

gcagttgtag cccgtaccgt tcgtcgtgtt ccgcctggcg caatcgaccc aactgttaaa 540

aatctgcagt ggggcgatct ggtacgcggt atgtttgaag ctgccgatcg tggtgctact 600

tatccgttcc tgacggatgg tgacgctcac ctgaccgagg gcgcaggcta caatatcgta 660

ctggtgcgta acggcgagct gcataccccg cgtcgtggcg ttctggaagg tattacccgt 720

cgtacggtgc tggagattgc agctgctcgc ggcctgaaaa ctcacgtcac cgaaatccca 780

atccaggccc tgtatgaatg cgacgaactg tttatgtgca gcaccgcggg cggtatcatg 840

ccactggtgc tgctggatgg caacattgta ggtgacggca ccgttggtcc ggtcacccgc 900

atgatttggg aagcgtactg ggacctgcac gacgacccgc agctgtctga accggtaacc 960

tatgcaccgc tcgag 975

<210> SEQ ID NO.4
<211> 325
<212> PRT
<213> Artificial design

<220>
<223> recombinant transaminase AH-TACM33 derived from Aspergillus terreus and Neptune silk Aeromonas

<400> 4

Met Ala Ser Met Asp Lys Val Phe Ala Gly Tyr Ala Ala Arg Gln Ala
1 5 10 15

Ile Leu Glu Ser Thr Glu Thr Thr Asn Pro Phe Ala Lys Gly Ile Ala
20 25 30

Trp Val Glu Gly Glu Leu Val Pro Leu Ala Glu Ala Arg Ile Pro Leu
35 40 45

Leu Asp Gln Gly Phe Met His Ser Asp Leu Thr Tyr Asp Val Pro Ser
50 55 60

Val Trp Asp Gly Arg Phe Phe Arg Leu Asp Asp His Ile Thr Arg Leu
65 70 75 80

Glu Ala Ser Cys Thr Lys Leu Arg Leu Arg Leu Pro Leu Pro Arg Asp
85 90 95

Gln Val Lys Gln Ile Leu Val Glu Met Val Ala Lys Ser Gly Ile Arg
100 105 110

Asp Ala Phe Val Glu Leu Ile Val Thr Arg Gly Leu Lys Gly Val Arg
115 120 125

Gly Thr Arg Pro Glu Asp Ile Val Asn Asn Leu Tyr Met Phe Val Gln
130 135 140

Pro Tyr Val Trp Val Met Glu Pro Asp Met Gln Arg Val Gly Gly Ser

145 150 155 160

Ala Val Val Ala Arg Thr Val Arg Arg Val Pro Pro Gly Ala Ile Asp
165 170 175

Pro Thr Val Lys Asn Leu Gln Trp Gly Asp Leu Val Arg Gly Met Phe
180 185 190

Glu Ala Ala Asp Arg Gly Ala Thr Tyr Pro Phe Leu Thr Asp Gly Asp
195 200 205

Ala His Leu Thr Glu Gly Ala Gly Tyr Asn Ile Val Leu Val Arg Asn
210 215 220

Gly Glu Leu His Thr Pro Arg Arg Gly Val Leu Glu Gly Ile Thr Arg
225 230 235 240

Arg Thr Val Leu Glu Ile Ala Ala Ala Arg Gly Leu Lys Thr His Val
245 250 255

Thr Glu Ile Pro Ile Gln Ala Leu Tyr Glu Cys Asp Glu Leu Phe Met
260 265 270

Cys Ser Thr Ala Gly Gly Ile Met Pro Leu Val Leu Leu Asp Gly Asn
275 280 285

Ile Val Gly Asp Gly Thr Val Gly Pro Val Thr Arg Met Ile Trp Glu
290 295 300

Ala Tyr Trp Asp Leu His Asp Asp Pro Gln Leu Ser Glu Pro Val Thr
305 310 315 320

Tyr Ala Pro Leu Glu
325

<210> SEQ ID NO.5
<211> 981
<212> DNA
<213> Aspergillus terreus

<400> 5
atggcaagca tggataaagt ttttgcaggt tatgcagcac gtcaggcaat tctggaaagc 60

accgaaacca ccaatccgtt tgcaaaaggt attgcatggg ttgaaggtga actggttccg 120

ctggcagaag cacgtattcc gctgctggat cagggtttta tgcatagcga tctgacctat 180

gatgttccga gcgtttggga tggtcgtttt tttcgtctgg atgatcatat tacccgtctg 240

gaagcaagct gtaccaaact gcgtctgcgt ctgccgctgc ctcgtgatca ggttaaacaa 300

attctggttg aaatggttgc caaaagcggt attcgtgatg catttgttga actgattgtt 360

acccgtggtc tgaaaggtgt tcgtggcacc cgtccggaag atattgttaa taatctgtat 420

atgtttgtgc agccgtatgt ttgggttatg gaaccggata tgcagcgtgt tggtggtagc 480

gcagttgttg cacgtaccgt tcgtcgtgtt ccgccgggtg caattgatcc gaccgttaaa 540

aatctgcagt ggggtgatct ggttcgtggt atgtttgaag cagcagatcg tggtgcaacc 600

tatccgtttc tgaccgatgg tgatgcacat ctgaccgaag gtagcggctt taacattgtt 660

ctggttaaag atggcgtgct gtatacaccg gatcgtggtg ttctgcaggg tgttacccgt 720

aaaagcgtta ttaatgcagc agaagccttt ggtattgaag ttcgtgttga atttgttccg 780

gttgaactgg catatcgctg tgatgaaatc tttatgtgta ccaccgcagg cggtattatg 840

ccgattacca ccctggatgg tatgccggtt aatggtggtc agattggtcc gattaccaaa 900

aaaatctggg atggttattg ggccatgcat tatgatgcag catatagctt tgaaattgat 960

tataatgaac gcaatctcga g 981

<210> SEQ ID NO.6
<211> 969
<212> DNA
<213> Neptune silk Aeromonas

<400> 6
atgctgacct ttcagaaagt tctgaccggt tttcagaccc gtgcagatgc acgtgcagaa 60

cgtaccgatg catttgcaga tggtattgca tggattgaaa atgaatttgt gccgattggc 120

aaagcacgta ttccgattct ggatcagggt tttctgcata gcgatctgac ctatgatgtt 180

ccggcagttt ggaatggtcg tatttttcgt ctggatgatc atctggatcg tctggaagtt 240

agctgtgcaa aaatgcgtct gccgctgccg attgcacgtc cggaactgcg tcgtctggtt 300

atggaactgg ttagccgtag cggtctgcgt gatgcctatg ttgaaattat tgttacccgt 360

ggcctgaaat ttctgcgtgg tgcacaggca gaagatatta ttccgaatct gtatctgatg 420

gccgttccgt atgtttggat tctgccgctg gaatatcaga atcatggtgc accggcagtt 480

gttacccgta ccgttcgtcg tacaccgccg ggtgcactgg atccgaccat caaaaatctg 540

cagtggggtg atctggttcg tggtctgatg gaagccggtg atcgtgatag ctttttccg 600

attctgccgg atggtgatgg taatgcaacc gaaggtgcag gctataacat tgttctggtt 660

cgtaatggcg aactgcatac accgcgtcgt ggtgttctgg aaggtattac ccgtcgtacc 720

gttctggaaa ttgcagcagc acgtggcctg aaaacacatg ttaccgaaat tccgattcag 780

gcactgtatg aatgtgatga actgtttatg tgtagcaccg caggcggtat tatgccgctg 840

gttctgctgg atggtaatat tgttggtgat ggcaccgttg gtccggttac ccgtatgatt 900

tgggaagcat attgggatct gcatgatgat ccgcagctga gcgaaccggt tacctatgca 960

ccgctcgag 969

<210> SEQ ID NO.7
<211> 33
<212> DNA
<213> Artificially synthesized

<220>
<223> primer designed as the transaminase gene of Aspergillus terreus.

<400> 7
ccgctcgagg ttacgctcgt tgtagtcaat ttc 33

<210> SEQ ID NO.8
<211> 29
<212> DNA
<213> Artificially synthesized

<220>
<223> primer designed as the transaminase gene of Aspergillus terreus.

<400> 8
ggaattccat atggcgtcta tggacaaag 29

<210> SEQ ID NO.9
<211> 30
<212> DNA
<213> Artificially synthesized

<220>
<223> primer designed as the transaminase gene of Neptune silk Aeromonas.

<400> 9
ccgctcgagc ggtgcatagg ttaccggttc 30

<210> SEQ ID NO.10
<211> 36
<212> DNA
<213> Artificially synthesized

<220>
<223> primer designed as the transaminase gene of Neptune silk Aeromonas.

<400> 10
ggaattccat atgctgacct tccaaaaagt actgac 36

<210> SEQ ID NO.11
<211> 27
<212> DNA
<213> Artificially synthesized

<220>
<223> antisense primer for amplification a fragment of transaminase gene derive from Aspergillus terreus.

<400> 11
gaacttcaga ccgcgggtga caatcag 27

<210> SEQ ID NO.12
<211> 23
<212> DNA
<213> Artificially synthesized

<220>
<223> sense primer for amplification a fragment of transaminase gene derive from Aspergillus terreus.

<400> 12
cacccgcggt ctgaagttcc tgc 23

<210> SEQ ID NO.13
<211> 24
<212> DNA
<213> Artificially synthesized

<220>
<223> antisense primer for amplification a fragment of transaminase gene derive from Aspergillus terreus.

<400> 13
cggcggaaca cgacgaacgg tacg 24

<210> SEQ ID NO.14
<211> 24
<212> DNA
<213> Artificially synthesized

<220>
<223> sense primer for amplification a fragment of transaminase gene derive from Aspergillus terreus.

<400> 14
ttcgtcgtac tccgccgggc gcac 24

<210> SEQ ID NO.15
<211> 25
<212> DNA
<213> Artificially synthesized

<220>
<223> antisense primer for amplification a fragment of transaminase gene derive from Aspergillus terreus.

<400> 15
tagcctgcgc cctcggtcag gtgag 25

<210> SEQ ID NO.16
<211> 25
<212> DNA
<213> Artificially synthesized

<220>
<223> sense primer for amplification a fragment of transaminase gene derive from Aspergillus terreus.

<400> 16

gaccgagggc gcaggctaca atatc 25

<210> SEQ ID NO.17
<211> 27
<212> DNA
<213> Artificially synthesized

<220>
<223> antisense primer for amplification a fragment of transaminase gene derive from Neptune silk Aeromonas.

<400> 17
cccttcagac cacgcgtaac gatgatc 27

<210> SEQ ID NO.18
<211> 27
<212> DNA
<213> Artificially synthesized

<220>
<223> sense primer for amplification a fragment of transaminase gene derive from Neptune silk Aeromonas.

<400> 18
ttacgcgtgg tctgaagggt gtgcgtg 27

<210> SEQ ID NO.19
<211> 28
<212> DNA
<213> Artificially synthesized

<220>
<223> antisense primer for amplification a fragment of transaminase gene derive from Neptune silk Aeromonas.

<400> 19
ccaggcggag tacgacgtac agtacgag 28

<210> SEQ ID NO.20
<211> 26
<212> DNA
<213> Artificially synthesized

<220>
<223> sense primer for amplification a fragment of transaminase gene derive from Neptune silk Aeromonas.

<400> 20
tacgtcgtgt tccgcctggc gcaatc 26

<210> SEQ ID NO.21
<211> 24
<212> DNA
<213> Artificially synthesized

<220>

<223> antisense primer for amplification a fragment of transaminase gene derive from Neptune silk Aeromonas.

<400> 21
gccgctgcct tccgtcgcgt tacc 24

<210> SEQ ID NO.22
<211> 25
<212> DNA
<213> Artificially synthesized

<220>
<223> sense primer for amplification a fragment of transaminase gene derive from Neptune silk Aeromonas.

<400> 22
gacggaaggc agcggcttca acatc 25

<210> SEQ ID NO.23
<211> 327
<212> PRT
<213> Aspergillus terreus

<400> 23

Met Ala Ser Met Asp Lys Val Phe Ala Gly Tyr Ala Ala Arg Gln Ala
1 5 10 15

Ile Leu Glu Ser Thr Glu Thr Thr Asn Pro Phe Ala Lys Gly Ile Ala
20 25 30

Trp Val Glu Gly Glu Leu Val Pro Leu Ala Glu Ala Arg Ile Pro Leu
35 40 45

Leu Asp Gln Gly Phe Met His Ser Asp Leu Thr Tyr Asp Val Pro Ser
50 55 60

Val Trp Asp Gly Arg Phe Phe Arg Leu Asp Asp His Ile Thr Arg Leu
65 70 75 80

Glu Ala Ser Cys Thr Lys Leu Arg Leu Arg Leu Pro Leu Pro Arg Asp
85 90 95

Gln Val Lys Gln Ile Leu Val Glu Met Val Ala Lys Ser Gly Ile Arg
100 105 110

Asp Ala Phe Val Glu Leu Ile Val Thr Arg Gly Leu Lys Gly Val Arg
115 120 125

Gly Thr Arg Pro Glu Asp Ile Val Asn Asn Leu Tyr Met Phe Val Gln
130 135 140

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Pro Tyr Val Trp Val Met Glu Pro Asp Met Gln Arg Val Gly Gly Ser
145                 150                 155                 160

Ala Val Val Ala Arg Thr Val Arg Arg Val Pro Pro Gly Ala Ile Asp
                165                 170                 175

Pro Thr Val Lys Asn Leu Gln Trp Gly Asp Leu Val Arg Gly Met Phe
            180                 185                 190

Glu Ala Ala Asp Arg Gly Ala Thr Tyr Pro Phe Leu Thr Asp Gly Asp
        195                 200                 205

Ala His Leu Thr Glu Gly Ser Gly Phe Asn Ile Val Leu Val Lys Asp
    210                 215                 220

Gly Val Leu Tyr Thr Pro Asp Arg Gly Val Leu Gln Gly Val Thr Arg
225                 230                 235                 240

Lys Ser Val Ile Asn Ala Ala Glu Ala Phe Gly Ile Glu Val Arg Val
                245                 250                 255

Glu Phe Val Pro Val Glu Leu Ala Tyr Arg Cys Asp Glu Ile Phe Met
            260                 265                 270

Cys Thr Thr Ala Gly Gly Ile Met Pro Ile Thr Thr Leu Asp Gly Met
        275                 280                 285

Pro Val Asn Gly Gly Gln Ile Gly Pro Ile Thr Lys Lys Ile Trp Asp
    290                 295                 300

Gly Tyr Trp Ala Met His Tyr Asp Ala Ala Tyr Ser Phe Glu Ile Asp
305                 310                 315                 320

Tyr Asn Glu Arg Asn Leu Glu
                325
```

<210> SEQ ID NO.24
<211> 323
<212> PRT
<213> Neptune silk Aeromonas

<400> 24

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Met Leu Thr Phe Gln Lys Val Leu Thr Gly Phe Gln Thr Arg Ala Asp
1               5                   10                  15

Ala Arg Ala Glu Arg Thr Asp Ala Phe Ala Asp Gly Ile Ala Trp Ile
            20                  25                  30
```

```
Glu Asn Glu Phe Val Pro Ile Gly Lys Ala Arg Ile Pro Ile Leu Asp
        35                  40                  45

Gln Gly Phe Leu His Ser Asp Leu Thr Tyr Asp Val Pro Ala Val Trp
    50                  55                  60

Asn Gly Arg Ile Phe Arg Leu Asp Asp His Leu Asp Arg Leu Glu Val
65                  70                  75                  80

Ser Cys Ala Lys Met Arg Leu Pro Leu Pro Ile Ala Arg Pro Glu Leu
                85                  90                  95

Arg Arg Leu Val Met Glu Leu Val Ser Arg Ser Gly Leu Arg Asp Ala
            100                 105                 110

Tyr Val Glu Ile Ile Val Thr Arg Gly Leu Lys Phe Leu Arg Gly Ala
        115                 120                 125

Gln Ala Glu Asp Ile Ile Pro Asn Leu Tyr Leu Met Ala Val Pro Tyr
    130                 135                 140

Val Trp Ile Leu Pro Leu Glu Tyr Gln Asn His Gly Ala Pro Ala Val
145                 150                 155                 160

Val Thr Arg Thr Val Arg Arg Thr Pro Pro Gly Ala Leu Asp Pro Thr
                165                 170                 175

Ile Lys Asn Leu Gln Trp Gly Asp Leu Val Arg Gly Leu Met Glu Ala
            180                 185                 190

Gly Asp Arg Asp Ser Phe Phe Pro Ile Leu Pro Asp Gly Asp Gly Asn
        195                 200                 205

Ala Thr Glu Gly Ala Gly Tyr Asn Ile Val Leu Val Arg Asn Gly Glu
    210                 215                 220

Leu His Thr Pro Arg Arg Gly Val Leu Glu Gly Ile Thr Arg Arg Thr
225                 230                 235                 240

Val Leu Glu Ile Ala Ala Ala Arg Gly Leu Lys Thr His Val Thr Glu
                245                 250                 255

Ile Pro Ile Gln Ala Leu Tyr Glu Cys Asp Glu Leu Phe Met Cys Ser
            260                 265                 270

Thr Ala Gly Gly Ile Met Pro Leu Val Leu Leu Asp Gly Asn Ile Val
        275                 280                 285
```

Gly Asp Gly Thr Val Gly Pro Val Thr Arg Met Ile Trp Glu Ala Tyr
290 295 300

Trp Asp Leu His Asp Asp Pro Gln Leu Ser Glu Pro Val Thr Tyr Ala
305 310 315 320

Pro Leu Glu

<210> SEQ ID NO.25
<211> 325
<212> PRT
<213> Artificial design

<220>
<223> Leu to Ile substitution at 38th of the transaminase AH-TACM32 derived from Aspergillus terreus and Neptune silk Aeromonas

<400> 25

Met Ala Ser Met Asp Lys Val Phe Ala Gly Tyr Ala Ala Arg Gln Ala
1 5 10 15

Ile Leu Glu Ser Thr Glu Thr Thr Asn Pro Phe Ala Lys Gly Ile Ala
20 25 30

Trp Val Glu Gly Glu Ile Val Pro Leu Ala Glu Ala Arg Ile Pro Leu
35 40 45

Leu Asp Gln Gly Phe Met His Ser Asp Leu Thr Tyr Asp Val Pro Ser
50 55 60

Val Trp Asp Gly Arg Phe Phe Arg Leu Asp Asp His Ile Thr Arg Leu
65 70 75 80

Glu Ala Ser Cys Thr Lys Leu Arg Leu Arg Leu Pro Leu Pro Arg Asp
85 90 95

Gln Val Lys Gln Ile Leu Val Glu Met Val Ala Lys Ser Gly Ile Arg
100 105 110

Asp Ala Phe Val Glu Leu Ile Val Thr Arg Gly Leu Lys Gly Val Arg
115 120 125

Gly Thr Arg Pro Glu Asp Ile Val Asn Asn Leu Tyr Met Phe Val Gln
130 135 140

Pro Tyr Val Trp Val Met Glu Pro Asp Met Gln Arg Val Gly Gly Ser
145 150 155 160

```
Ala Val Val Ala Arg Thr Val Arg Arg Thr Pro Pro Gly Ala Leu Asp
                165                 170                 175

Pro Thr Ile Lys Asn Leu Gln Trp Gly Asp Leu Val Arg Gly Leu Met
            180                 185                 190

Glu Ala Gly Asp Arg Asp Ser Phe Phe Pro Ile Leu Pro Asp Gly Asp
        195                 200                 205

Gly Asn Ala Thr Glu Gly Ala Gly Tyr Asn Ile Val Leu Val Arg Asn
    210                 215                 220

Gly Glu Leu His Thr Pro Arg Arg Gly Val Leu Glu Gly Ile Thr Arg
225                 230                 235                 240

Arg Thr Val Leu Glu Ile Ala Ala Ala Arg Gly Leu Lys Thr His Val
                245                 250                 255

Thr Glu Ile Pro Ile Gln Ala Leu Tyr Glu Cys Asp Glu Leu Phe Met
            260                 265                 270

Cys Ser Thr Ala Gly Gly Ile Met Pro Leu Val Leu Leu Asp Gly Asn
        275                 280                 285

Ile Val Gly Asp Gly Thr Val Gly Pro Val Thr Arg Met Ile Trp Glu
    290                 295                 300

Ala Tyr Trp Asp Leu His Asp Asp Pro Gln Leu Ser Glu Pro Val Thr
305                 310                 315                 320

Tyr Ala Pro Leu Glu
                325
```

Claims

1. A transaminase or a modified compound, functional equivalent, functional fragment or variant thereof, wherein an amino sequence of the transaminase comprises a sequence selected from one of the following sequences:

   a) an amino acid sequence as shown in SEQ ID NO: 2 or 4;
   b) an amino acid sequence with at least 80% identity to the amino acid sequence as shown in SEQ ID NO: 2 or 4 and having the activity of an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the amino acid sequence is not an amino acid sequence encoded by a nucleotide sequence as shown in SEQ ID NO: 5 or 6;
   c) a protein which is derived from SEQ ID NO: 2 or 4 by subjecting the amino acid sequence as shown in SEQ ID NO: 2 or 4 to substitution, deletion or addition one or more amino acids, and having the activity of an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the amino acid sequence is not the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 5 or 6, wherein the high stereoselective refers to the content of one of the stereoisomers being at least about 1.1 times that of the other.

2. The transaminase according to claim 1, wherein the amino acid sequence of the transaminase is an amino acid sequence acquired by substituting leucine at the 38th site of the amino acid sequence as shown in SEQ ID NO: 2 by isoleucine.

3. A nucleotide, wherein the nucleotide encodes the transaminase or the modified compound, functional equivalent, functional fragment or variant thereof according to claim 1 or 2.

4. The nucleotide according to claim 3, wherein a sequence of the nucleotide includes a sequence selected from one of the following sequences:

a) a nucleotide sequence as shown in SEQ ID NO: 1 or 3;
b) a nucleotide sequence with at least 80% identity to the nucleotide sequence as shown in SEQ ID NO: 1 or 3 and encoding an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the nucleotide sequence is not the nucleotide sequence as shown in SEQ ID NO: 5 or 6;
c) a nucleotide sequence capable of hybridizing with the nucleotide sequence as shown in SEQ ID NO: 1 or 3 under highly stringent conditions and encoding an omega-transaminase with high stereoselective R-configuration catalytic activity, wherein the nucleotide sequence is not the nucleotide sequence as shown in SEQ ID NO: 5 or 6,

wherein the high stereoselective refers to the content of one of the stereoisomers being at least about 1.1 times that of the other.

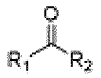
5. A recombinant vector, wherein the nucleotide according to claim 3 or 4 is effectively connected in the recombinant vector.

6. The recombinant vector according to claim 5, wherein the recombinant vector is pET22b-CM32 or pET22b-CM33.

7. A host cell, wherein the recombinant vector according to claim 5 or 6 is transformed or transfected into the host cell.

8. A method for synthesizing a chiral amine, wherein the method comprises the following steps: making a ketone compound, the transaminase or the modified compound, functional equivalent, functional fragment or variant thereof according to claim 1, pyridoxal phosphate, and an amino donor to react in a reaction system so as to obtain the chiral amine of R configuration.

9. The method according to claim 8, wherein the ketone compound is



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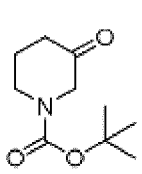
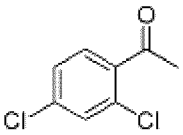
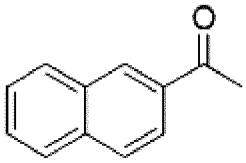
wherein R_1 and R_2 are independently C_1 to C_8 alkyl, C_5 to C_{10} naphthenic base, C_5 to C_{10} aryl or C_5 to C_{10} heteroaryl; or R_1 and R_2 form a C_5 to C_{10} heterocyclic radical, a C_5 to C_{10} carbocyclic radical or C_5 to C_{10} heteroaryl with a carbon on a carbonyl group; heteroatoms in the C_5 to C_{10} heterocyclic radical and C_5 to C_{10} heteroaryl are independently selected from at least one of nitrogen, oxygen and sulfur; the aryl in the C_5 to C_{10} aryl, the heteroaryl in the C_5 to C_{10} heteroaryl, the carbocyclic radical in the C_5 to C_{10} carbocyclic radical or the heterocyclic radical in the C_5 to C_{10} heterocyclic radical is independently unsubstituted or is substituted by at least one radical of halogen, alkoxy or alkyl; preferably, the ketone compound

is selected from



,


or


.

10. The method according to claim 8 or 9, wherein the reaction system further includes a dissolution promoter, and the dissolution promoter is dimethyl sulfoxide or polyethylene glycol, and the polyethylene glycol is preferably PEG-400.

11. The method according to claim 9, wherein the C1 to C8 alkyl is C1 to C8 linear alkyl, the C5 to C10 heteroaryl is a pyridine group, the alkoxy is C1 to C6 alkoxy, the heterocyclic radical in the C5 to C10 heterocyclic radical is piperidine, a substituent on the aryl in the C5 to C10 aryl, the heteroaryl in the C5 to C10 heteroaryl, the carbocyclic radical in the C5 to C10 carbocyclic radical or the heterocyclic radical in the C5 to C10 heterocyclic radical is independently C1 to C6 linear alkyl or C1 to C6 alkoxy, and the amino donor is isopropylamine or D-alanine.

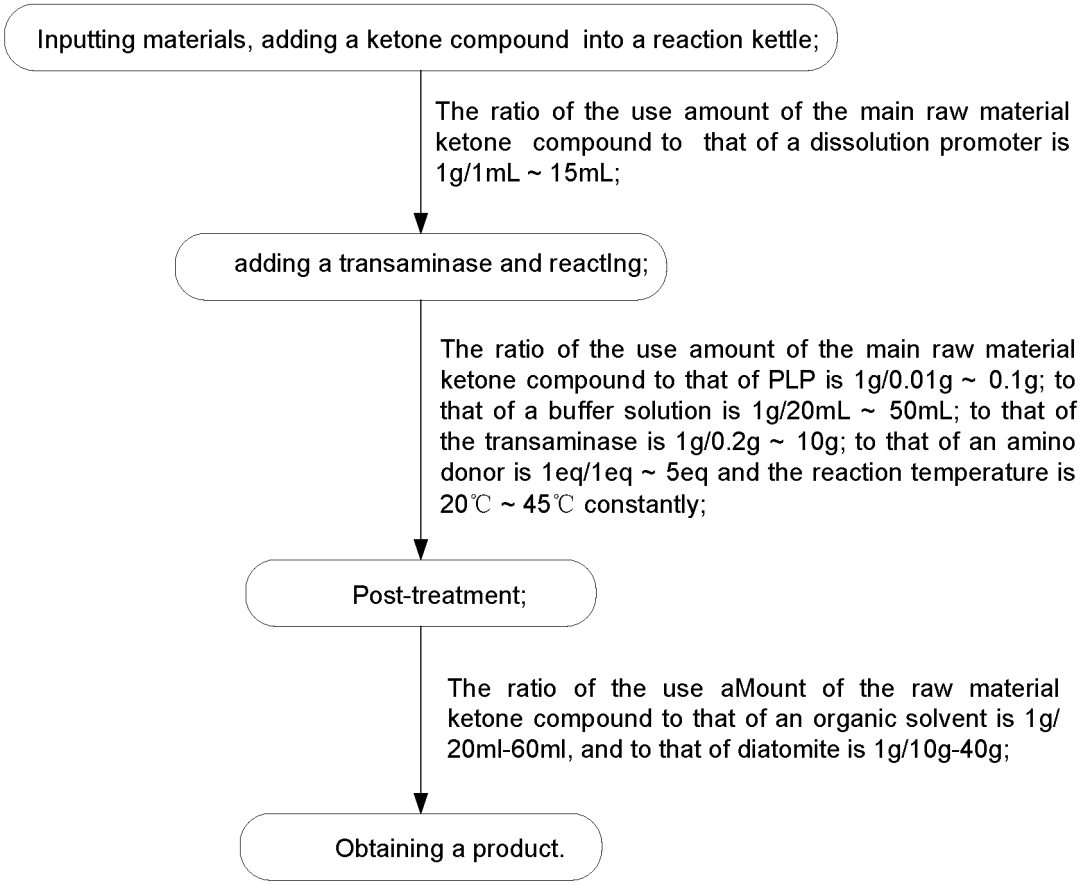

Inputting materials, adding a ketone compound into a reaction kettle;
The ratio of the use amount of the main raw material ketone compound to that of a dissolution promoter is 1g/1mL ~ 15mL;
adding a transaminase and reactIng;
The ratio of the use amount of the main raw material ketone compound to that of PLP is 1g/0.01g ~ 0.1g; to that of a buffer solution is 1g/20mL ~ 50mL; to that of the transaminase is 1g/0.2g ~ 10g; to that of an amino donor is 1eq/1eq ~ 5eq and the reaction temperature is 20℃ ~ 45℃ constantly;
Post-treatment;
The ratio of the use aMount of the raw material ketone compound to that of an organic solvent is 1g/20ml-60ml, and to that of diatomite is 1g/10g-40g;
Obtaining a product.

Fig. 1

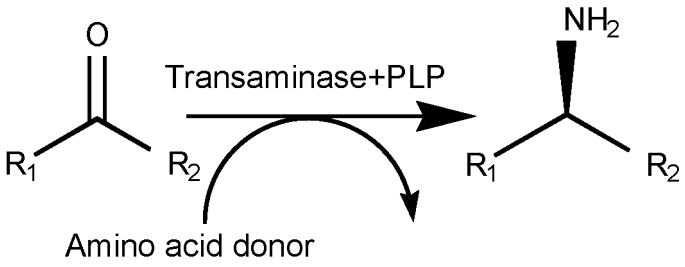

O
R1
R2
Transaminase+PLP
Amino acid donor
NH2
R1
R2

Fig. 2

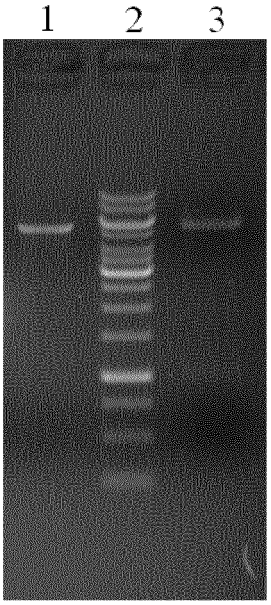


Fig. 3

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taAT.seq          MASMDKVFACYAARQAILESTETTNPFAKGIAWVEGELVPLAEARIPLIDQGFMHSDLTY    60
AH-TACM33.seq     MASMDKVFACYAARQAILESTETTNPFAKGIAWVEGELVPLAEARIPLIDQGFMHSDLTY    60
taHN.seq          MLTFQKVLTCFQTRAD..ARAERTDAFADGIAWIENEFVPIGKARIPIIDQGFLHSDLTY    58
Consensus         m    kv  g   r       e t  fa giaw e e vp   arip ldqgf hsdlty

taAT.seq          DVPSVWDGRFFRLDDHITRLEASCTKLRIRLPLPRDQVKQILVEMVAKSGIRDAFVELIV    120
AH-TACM33.seq     DVPSVWDGRFFRLDDHITRLEASCTKLRIRLPLPRDQVKQILVEMVAKSGIRDAFVELIV    120
taHN.seq          DVPAVWNGRIFRLDDHLDRLEVSCAKMRIPLPIARPEIRRLVMEIVSRSGIRDAYVEIIV    118
Consensus         dvp vw gr frlddh  rle sc k rl lp  r        e v  sg rda ve iv

taAT.seq          TRGLKGVRCIRPEDIVNNLYMFVQPYVWVMEPDMQRVCGSAVVARTVRRVPPGAIDPTVK    180
AH-TACM33.seq     TRGLKGVRCIRPEDIVNNLYMFVQPYVWVMEPDMQRVCGSAVVARTVRRVPPGAIDPTVK    180
taHN.seq          TRGLKFIRCAQAEDIIPNLYLMAVPYVWILPLEYQNHCAPAVVTRTVRRTPPGALDPTIK    178
Consensus         trglk  rg   edi  nly    pyvw      q  g  avv rtvrr ppga dpt k

taAT.seq          NLQWGDIVRCMFEAADRGATYPFLTDGDAHLIECSCFNIVLVKDGVLYIPDRGVLQGVTR    240
AH-TACM33.seq     NLQWGDIVRCMFEAADRGATYPFLTDGDAHLIECACYNIVLVRNGELHIPRRGVLEGITR    240
taHN.seq          NLQWGDIVRCLMEAGDRDSFFPILPDGDGNAIECACYNIVLVRNGELHIPRRGVLEGITR    238
Consensus         nlqwgdlvrg  ea dr    p l dgd   teg g nivlv  g l tp rgvl g tr

taAT.seq          KSVINAAEAFCIEVRVEFVPVELAYRCDEIFMCTTAGGIMPITTLDGMPVNGGQIGPITK    300
AH-TACM33.seq     RTVLEIAAARCLKTHVTEIPIQALYECDELFMCSTAGGIMPLVLLDGNIVGDGTVGPVTR    300
taHN.seq          RTVLEIAAARCLKTHVTEIPIQALYECDELFMCSTAGGIMPLVLLDGNIVGDGTVGPVTR    298
Consensus           v   a a g    v   p    y cde fmc taggimp   ldg  v  g  gp t

taAT.seq          KIWDGYWAMTYDAAYSFEIDYNERNL                                    326
AH-TACM33.seq     MIWEAYWDLHDDPQLSEPVTYAP..L                                    324
taHN.seq          MIWEAYWDLHDDPQLSEPVTYAP..L                                    322
Consensus          iw  yw  h d   s    y   l
```

Fig. 4

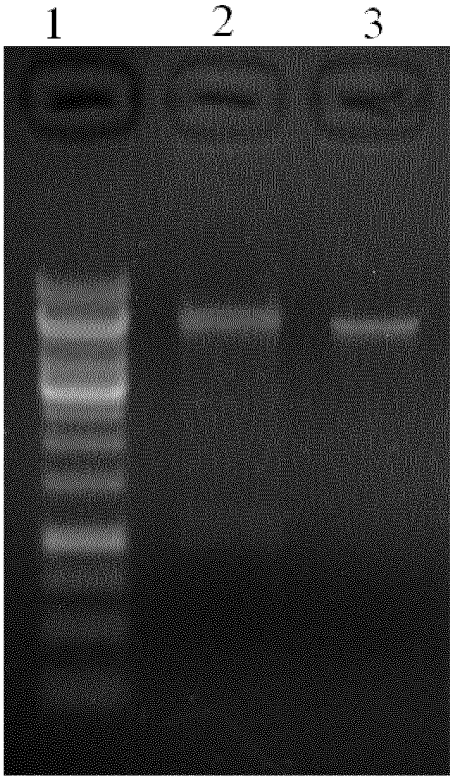

Fig. 5

```
taAT.seq          MASMDKVFAQYAAEQAILESTETTNPFAKGIAWVPGDLVELAERARIPLIDQGEMHSDITY   60
AH-TACM32.seq     MASMDKVFAQYAAEQAILESTETTNPFAKGIAWVPGDLVELAERARIPLIDQGEMHSDITY   60
taHN.seq          MLTFQRVLTQFQTEAD..ARAERTDAFAIGIAWIPNPFVEIGKARIPIIDQGELHSDITY   58
Consensus         m    kv  g   r       e t  fa giaw e e vp   arip ldqgf hsdlty

taAT.seq          DVESVWDGRRFRLDDFIITPLEASCTKLRIRLPLPRDQVEQILVDMVAKSGIRDAFVELIV   120
AH-TACM32.seq     DVESVWDGRRFRLDDFIITPLEASCTKLRIRLPLPRDQVEQILVDMVAKSGIRDAFVELIV   120
taHN.seq          DVEAVWNGRTFRLDDFILDPLEVSCAQMRIPLPIAEPELPRLVMELVSRSGIRDAYVEIIV   118
Consensus         dvp vw gr frlddh  rle sc k rl lp  r        e v  sg rda ve iv

taAT.seq          TRGLKGVRGIRPEDIVNNIYMFVCPYVWVMEPDMQRVGGSAVVARTVRPVPPGAIDPTVK   180
AH-TACM32.seq     TRGLKGVRGIRPEDIVNNIYMFVCPYVWVMEPDMQRVGGSAVVARTVRPTPPGALDPTIK   180
taHN.seq          TRGLKFLRGAQAEDIIPNIYLMAVPYVWILPLEYQNHGAPAVVTRTVRPTPPGALDPTIK   178
Consensus         trglk  rg   edi  nly    pyvw      q  g  avv rtvrr ppga dpt k

taAT.seq          NLQWGDLVRGMFEAADRGATYPFLTDGDAHLTEGSGFNIVLVKDGVLYIPDRGVLQGVTR   240
AH-TACM32.seq     NLQWGDLVRGLMEAGDRDSFFPILPDGDGNATEGAGYNIVLVRNGELHIPRGVLEGITR   240
taHN.seq          NLQWGDLVRGLMEAGDRDSFFPILPDGDGNATEGAGYNIVLVRNGELHIPRGVLEGITR   238
Consensus         nlqwgdlvrg  ea dr    p l dgd   teg g nivlv  g l tp rgvl g tr

taAT.seq          KSVINAAEAKGIEVPVEFVPVELAMRCDEIFMCTAGGIMEITTLDGMPVNGGQIGPITK   300
AH-TACM32.seq     RTVLEIAAAPGLKTHVTEIPIQALMECDEIFMCSTAGGIMELVLLDGNIVGDGTVGPVTR   300
taHN.seq          RTVLEIAAAPGLKTHVTEIPIQALMECDEIFMCSTAGGIMELVLLDGNIVGDGTVGPVTR   298
Consensus           v   a a g    v   p    y cde fmc taggimp   ldg  v  g  gp t

taAT.seq          KIWDGYWAMFYDAAYSFEIDYNERNL                                    326
AH-TACM32.seq     MIMEAYWDLFDDPQISEPVTYAPLE.                                    325
taHN.seq          MIMEAYWDLFDDPQISEPVTYAPLE.                                    323
Consensus          iw  yw  h d   s    y
```

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2014/090080

A. CLASSIFICATION OF SUBJECT MATTER

C12N 9/10 (2006.01) i; C12N 15/54 (2006.01) i; C12P 17/12 (2006.01) i; C12P 13/00 (2006.01) i; C12R 1/66 (2006.01) i; C12R 1/01 (2006.01) i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N; C12P; C12R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CPRSABS; CNABS; DWPI; SIPOABS; CNTXT; EPTXT; WOTXT; USTXT; CATXT; GBTXT; JPTXT; KRTXT; CNKI; ISI; PUBMED; GOOGLE; ELSEVIER: Hyphomonas neptunium, Aspergillus terreus, terreus, neptunium, transaminase, omega-transaminase, ω-transaminase, bacterium, bacteria, molecular modification, molecular designing, mutate, mutation, chiral amine, optical activity, synthesize, synthesis, asymmetric synthesis; GenBank; DDBJ; EMBL: sequences search on SEQ ID NOs: 1-6.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 102482650 A (LONZA AG) 30 May 2012 (30.05.2012) see the abstract, claims, see the embodiments 1-4, SEQ ID NOs: 3-4	1, 3-11
A	Cassimjee KE et al. "Chromobacterium violaceum ω-transaminase variant Trp60Cys shows increased specificity for (S)-1-phenylethylamine and 4'-substituted acetophenones, and follows Swain-Lupton parameterization" Organic & Biomolecular Chemistry, vol. 28, no. 10, 28 July 2012 (28.07.2012), pages 5466-5470, see the abstract	2
A	WO 2009088949 A1 (VERENIUM CORP et al.) 16 July 2009 (16.07.2009) see the whole document	1-11
A	CN 101512005 A (LONZA AG) 19 August 2009 (19.08.2009) see the whole document	1-11

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2015	10 February 2015
Name and mailing address of the ISA State Intellectual Property Office of the P. R. China No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088, China Facsimile No. (86-10) 62019451	Authorized officer LI, Xiaoqu Telephone No. (86-10) 62089432

Form PCT/ISA /210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2014/090080

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1818411 A1 (LONZA AG) 15 August 2007 (15.08.2007) see the whole document	1-11
A	JIA, Honghua et al. "Progress in ω-transaminase in chiral amine synthesis" Modern Chemical Industry, vol. 32, no. 3, 31 March 2012 (31.03.2012), pages 16-22, see the whole document	1-11
A	Jong-Shik Shin and Byung-Gee Kim. "Asymmetric Synthesis of Chiral Amines with ω-Transaminase " Biotechnology and Bioengineering, vol. 65, no. 2, 20 October 1999 (20.10.1999), pages 206-211, see the whole document	8-11
A	Jong-Shik Shin and Byung-Gee Kim. "Comparison of the ω-Transaminase from Different Microorganisms and Application to Production of Chiral Amines" Biosci. Biotechnol. Biochem., vol. 65, no. 8, 31 August 2001 (31.08.2001) pages 1782-1788, see the whole document	1-11

Form PCT/ISA /210 (continuation of second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2014/090080

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

[1] Invention 1: claims 1, 3-7 (all partially) and 2, relate to a transaminase comprising a sequence of SEQ ID NO: 2, a modification, functional equivalent, functional fragment or variant thereof, a nucleotide sequence encoding the transaminase, a recombinant vector comprising the nucleotide sequence and a host cell comprising the recombinat vector;

[2] Invention 2: claims 1, 3-7 (all partially), relate to a to a transaminase comprising a sequence of SEQ ID NO: 4, a modifition, functional equivalent, functional fragment or variant thereof, a nucleotide sequence encoding the transaminase, a recombinant vector comprising the nucleotide sequence and a host cell comprising the recombinat vector;

[3] Invention 3: claims 9-11, relate to a method of synthesis of chiral amine.

[4] D1: CN102482650 A (LONZA AG), 30.May 2012 (30.05.2012), see the abstract, the claims, SEQ ID NO:3.

[5] The common or corresponding technical features between inventions 1 and 2 are the function of amino acid sequences as (R)-selective ω-transaminase showing highly specificity, and the common or corresponding technical features among inventions 1-2 and 3 are transaminases, modifications, functional equivalents, functional fragment or variants thereof. D1 discloses a sequence of (R)-selective ω-transaminase as shown in SEQ ID NO:3 which is 83% identify to SEQ ID NO: 4 of the present application(see the abstract, claims, SEQ ID NO:3). Thus, D1 discloses the amino acid sequence as (R)-selective ω-transaminase showing highly specificity and a transaminase, a modifications, a functional equivalent, a functional fragment or variant thereof. Thus, inventions 1 and 2, inventions 1-2 and 3 lack common or corresponding special technical features, and inventions 1-3 are not so linked as to form a single general inventive concept and therefore do not meet the requirements of unity of invention as defined in PCT rule 13.1 and 13.2

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA /210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2014/090080

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
WO 2009088949 A1	16 July 2009	EP 2238242 A4	11 January 2012
		JP 2011514141 A	06 May 2011
		AU 2008347234 A1	16 July 2009
		CN 101965397 A	02 February2011
		EP 2238242 A1	13 October 2010
		JP 5563990 B2	30 July 2014
		JP 2014209913 A	13 November 2014
		US 8709772 B2	29 April 2014
		EP 2238242 B1	26 November 2014
		US 2011033391 A1	10 February 2011
		US 2014165221 A1	12 June 2014
		CA 2710683 A1	16 July 2009
CN 101512005 A	19 August 2009	EA 016471 B1	30 May 2012
		ES 2474691 T3	09 July 2014
		KR 20090050054 A	19 May 2009
		JP 2010502202 A	28 January 2010
		CA 2659300 A1	13 March 2008
		EP 2064331 B1	02 April 2014
		EP 1897956 A1	12 March 2008
		CA 2659300 C	18 February 2014
		KR 101119501 B1	28 February 2012
		CN 101512005 B	02 January 2013
		US 2009325224 A1	31 December 2009
		IL 196779 D0	01 August 2011
		EP 2064331 A1	03 June 2009
		EA 200970247 A1	28 August 2009
		AU 2007294090 B2	05 July 2012
		BR PI0716874 A2	15 October 2013

Form PCT/ISA /210 (patent family annex) (July 2009)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2014/090080

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
		JP 5102297 B2	19 December 2012
		MX 2009002392 A	20 March 2009
		WO 2008028654 A1	13 March 2008
		AU 2007294090 A1	13 March 2008
		US 8728750 B2	20 May 2014
CN 102482650 A	30 May 2012	US 2012156706 A1	21 June 2012
		WO 2011026556 A1	10 March 2011
		EP 2473601 A1	11 July 2012
		AU 2010291586 A1	01 March 2012
		KR 20120096465 A	30 August 2012
		EA 201270350 A1	30 July 2012
		SG 178842 A1	27 April 2012
		MX 2012002561 A	10 April 2012
		CA 2772426 A1	10 March 2011
		IL 218397 D0	30 April 2012
		US 8577622 B2	05 November 2013
		CN 103472093 A	25 December 2013
EP 1818411 A1	15 August 2007	DK 1987152 T3	30 August 2010
		EP 1987152 B1	26 May 2010
		AU 2007214717 B2	22 December 2011
		EA 017070 B1	28 September 2012
		JP 2009526531 A	23 July 2009
		IL 219163 D0	28 June 2012
		IL 219164 D0	28 June 2012
		JP 5430945 B2	05 March 2014
		DE 602007006765 D1	08 July 2010
		IL 192637 D0	11 February 2009
		AU 2007214717 A1	23 August 2007

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2014/090080

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
		PT 1987152 E	18 August 2010
		CN 101384723 B	27 March 2013
		AT 469233 T	15 June 2010
		BRPI 0707454 A2	03 May 2011
		CA 2637821 A1	23 August 2007
		CN 101384723 A	11 March 2009
		EP 2202318 A1	30 June 2010
		EP 1987152 A1	05 November 2008
		JP 2013188217 A	26 September 2013
		EP 2202317 A1	30 June 2010
		KR 20080093437 A	21 October 2008
		WO 2007093372 A1	23 August 2007
		KR 101294499 B1	07 August 2013
		MX 2008010416 A	27 January 2009
		IL 192637 A	31 December 2014
		US 2009246837 A1	01 October 2009
		EA 200870262 A1	27 February 2009
		ES 2345734 T3	30 September 2010

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REFERENCES CITED IN THE DESCRIPTION

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Non-patent literature cited in the description

- **RENAT KADYROV et al.** Highly Enantioselective Hydrogen-Transfer Reductive Amination: Catalytic Asymmetric Synthesis of Primary Amines. *Angewandte Chemie International Edition,* 2003, vol. 42 (44), 5472-5474 **[0003]**
- **OHKUMA T et al.** Trans-RuH (eta1-BH4) (binap) (1,2-diamine): a catalyst for asymmetric hydrogenation of simple ketones under base-free conditions. *Journal of the American Chemical Society,* 2002, vol. 124 (23), 6508-6509 **[0003]**